2140

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SCIENTIFIC PROCEEDINGS.

New York Meeting.

New York Post-Graduate Hospital, October 20, 1926.

3204

Production by Digitalis of T-Wave Changes Similar to Those of Coronary Occlusion.

A. C. DeGRAFF AND C. L. WIBLE.

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The T-wave change which occurs with clinical coronary occlusion[1] was recently confirmed by experimental coronary occlusion in the cat.[2] It was suggested at that time that it might be possible to produce this characteristic peculiarity of the T-wave by some means other than coronary occlusion. In the course of another investigation we have found this type of T-wave to occur in the cat after the administration of digitalis.

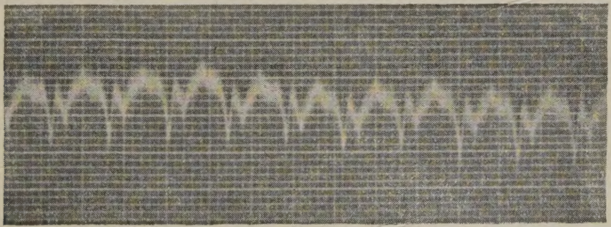

FIGURE 1.

Figure 1 shows a control electrocardiogram before the administration of digitalis. When a little over one-half the lethal dose had been injected into the cat, and electrocardiogram such as Figure 2 was obtained. This tracing definitely shows a T-wave coming off the S-wave before the base line is reached. Except for the fact that the T-wave comes off the S-wave instead of the R, it is precisely the type of curve described as characteristic of left coronary occlusion.

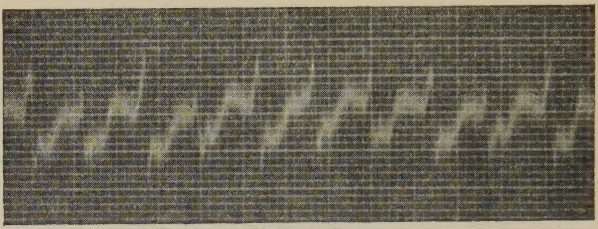

FIGURE 2.

This type of T-wave is by no means the normal type produced by digitalis, but it is interesting to note that digitalis can produce it.

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[1] Rothschild, M. A., Mann, H., and Oppenheimer, B. S., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 253.

[2] Cold, H., DeGraff, A. C., and Edwards, D. J., *ibid.*, 1926, xxiii, 664.

3205

The Relation Between the Virus of Epithelioma Contagiosum and the Vaccine Virus.

HOWARD B. ANDERVONT. (Introduced by Charles E. Simon.)

From the Department of the Filterable Viruses of the School of Hygiene and Public Health of the Johns Hopkins University, Baltimore, Maryland.

As the result of a recent study of the relationship existing between the virus of *epithelioma contagiosum* and the vaccine virus, the author concludes that while the vaccine virus is definitely pathogenic for the fowl, the *epithelioma contagiosum* virus is only mildly pathogenic for the rabbit. The lesions which the vaccine virus evokes in the fowl are characteristic of vaccinia, while those which the *epithelioma contagiosum* virus produces in the rabbit, are not specific. While the vaccine virus will give rise to the formation of typical Guarnieri bodies both in the skin and in the cornea of the inoculated fowl, the *epithelioma contagiosum* virus produces no Bollinger bodies in either the skin or the cornea of the inoculated rabbit. It is especially noteworthy that the fowl's skin and cornea are capable of producing both Guarnieri and Bollinger bodies and that they respond to the introduction of the two viruses in question in a perfectly specific manner.

Fowls develop a definite and specific immunity to both the virus of *epithelioma contagiosum* and to the vaccine virus, but a preliminary infection with either does not protect against a subsequent infection with the other. A preliminary inoculation of the rabbit with the *epithelioma contagiosum* virus will similarly not protect the animal against a subsequent infection with the vaccine virus, and a previous infection with the vaccine virus is without effect against the slight response which the rabbit may normally offer to the *epithelioma* virus. The rabbit responds to infection with the vaccine virus with the production of virudicidal antibodies against that virus, which appear free in the blood; such blood, however, is without manifest effect upon the *epithelioma contagiosum* virus. The fowl is apparently incapable of producing either free or sessile antibodies against either the vaccine virus or the *epithelioma contagiosum* virus.

The author's results support the claims of Loewenthal and his coworkers[1] regarding the lack of identity or even of a close relationship between the vaccine virus and the virus of *epithelioma contagiosum*, and stand in opposition to those of Van Heelsbergen[2] and Toyoda.[3]

The experiments were conducted with two strains of the *epithelioma contagiosum* virus, one obtained from Dr. J. R. Beach of Berkeley, California, and the other from Dr. O. de Blieck of Utrecht, through Dr. H. A. Gins of the *Koch institute für Infektionskrankheiten.*

Of the vaccine virus four samples were available, all of which were obtained from Messrs. H. K. Mulford and Company of Glenolden, Pennsylvania.

Since the conclusion of the author's experiments, a note by J. Basset[5] and one by G. Blanc and C. Melanidi[4] have appeared, in which the writers, the last mentioned working with the American strain of the *epithelioma contagiosum* virus from Dr. Simon's laboratory at the School of Hygiene and Public Health of the Johns Hopkins University, with which the author's experiments were in part carried on, likewise arrive at the conclusion that the virus of *epithelioma contagiosum* and of *vaccinia* are not identical.

---

[1] Loewenthal, W., Kadowaki, Y., and Kondo, S., *Centbl. Bakt. Parasit. u. Infektk.*, 1925, xciv, 185-200.

[2] van Heelsbergen, T. 1920. Reprint from Tijdsch. vergelijk, geneesk., 3 Afl. 3, 1-35.

[3] Toyoda, T., *Zeitsch. Hyg., u. Infektk.*, 1924, cii, 592-612.

[4] Blanc, G., and Melanidi, C., *Comptes rend. de la Soc. de Biol.*, 1926, xciv, 825.

[5] Basset, J., 1926, *ibid.*, 525.

3206

A Preliminary Study of Leonurus Sibiricus (I-Mu-Tsao).*

FU-TANG CHU, SHUN-MING CHEN AND K. K. CHEN.

From the University of Wisconsin, Madison, Wis.

The seeds and stems of *Leonurus sibiricus L.* or I-Mu-Tsao (meaning "grass to benefit the mother" in Chinese) are widely used in China for post-partum hemorrhages and menstrual disorders.[1] Peckolt-Rio[2] states that the juice of the leaves is good for hemoptysis, and an infusion for hysteria and menstrual troubles. The same author succeeded in isolating a crystalline substance from the leaves which he called *leonurin.* No chemical or pharmacological study was made with this substance.

Our study is pharmaco-chemical in nature. An intravenous injection in dogs of 2 to 5 cc. of a 10 per cent decoction (*i. e.*, each 10 gm. of material to 100 cc. of water), made from the leaves and stem, resulted in a brief fall of blood pressure, decrease in kidney volume, contraction of uterus, and stimulation of intestinal peristalsis. An attempt was then made to isolate any active principle by percolating the powdered leaves and stems with alcohol, distilling off the latter from the percolate under reduced pressure, and removing the ordinary plant constituents (resins, pigments, etc.) by lead acetate, the excess of which was precipitated with dilute sulphuric acid. When the filtrate is concentrated, a crystalline mass is obtained, which upon purification with alcohol produces the same physiological effects as the decoction. Half of a gram of this substance kills an anesthetized dog of 7.7 kilos by circulatory collapse. Qualitative chemical analyses show that these crystals are mostly potassium sulphate and potassium chloride, and that the parts of the plant under investigation contain no alkaloids. If the lead acetate in the percolate is removed by hydrogen sulphide instead of sulphuric acid, and the filtrate upon concentration is shaken with ether continuously for 15 hours, the ether extract after evaporation gives rise to a residue containing needle-like crystals. This residue is pungent in odor, soluble in dilute alcohol (about 12 per cent by volume), and contains no potassium salts. When injected into anesthetized dogs, it constricts the renal vessels and lowers the blood pressure slightly. The yield from 1.5 kilograms of the crude material is so small that no further study is possible.

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* This work was planned at Peking Union Medical College, Peking, China, and concluded by K. K. Chen at the University of Wisconsin, Madison, Wisconsin.

The impression of this preliminary investigation is that the leaves and stems of *Leonurus sibiricus* probably contain an organic principle, producing renal vasoconstriction and a slight fall of blood pressure. Further experimentation is necessary for its isolation and action. The richness of potassium salts in the plant should be borne in mind.

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[1] Li, Shih-cheng, *Pentsao Kang Mu*, 1596, chap. xv.

[2] Peckolt-Rio, Th., *Ber. pharmac. Gesell.*, 1904, xiv, 384.

3207

Detoxification of and the Immunity Production to Ricin by Sodium Ricinoleate.

EMMETT B. CARMICHAEL. (Introduced by Shiro Tashiro.)

From the Department of Biochemistry, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

The works of Larson[1] and his co-workers on sodium ricinoleate suggested the possibility of its use with other toxins such as those of plants and animals. Ricin was chosen first because the author, during the studies on the chemical basis of its toxicity, found that certain chemical and physical treatments could not only detoxify the toxin, but would produce an immunity to ricin in the animals when so treated.[2]

The ricin was prepared by the method of Osborne, Mendel and Harris.[3] A fresh aqueous solution was made for each experiment. It was clear and light yellow in color when concentrated.

The sodium ricinoleate was kindly furnished by Professor Larson. A 10 per cent stock solution was made in distilled water in a pyrex flask. It was light yellow in color.

These two solutions were mixed in such proportions as to make about 3 per cent of sodium ricinoleate in final volume. When the solution of ricin, containing 2,000 lethal doses for a rabbit in 5 to 10 cubic centimeters, was mixed with the sodium ricinoleate, it immediately became milky and opaque.

The mixed solutions were allowed to stand for one hour and then were injected into rabbits subcutaneously. The doses of the toxin employed varied over a rather wide range. The typical results obtained are given in the following table.

TABLE I.

Rabbit No.	Weight	Lethal doses* of Ricin	Sodium Ricinoleate
52	1850	1000	0.5 gm.
53	1875	400	0.2 gm.
54	1600	2000	0.4 gm.
55	2900	2000	0.2 gm.

* (0.05 mg. is a lethal dose for a rabbit.)

An inspection of the table makes it obvious that it is possible to inject a large number of lethal doses of ricin, if mixed with sodium ricinoleate, without killing the animal. The animals injected with 400 or more lethal doses of the treated ricin always showed an ulcer at the site of the injection. The minimum dose that will cause an ulcer has not been determined.

The detoxification of the ricin must have been complete or nearly so, since such large numbers of lethal doses have been given successfully without causing death. Each lethal dose now in the presence of this soap must have, at the maximum, less than 1/2000 of its original toxicity remaining since the animal lived when 2000 lethal doses were injected. No attempt has yet been made to determine the maximum amount of the mixture of the toxin and sodium ricinoleate that can be injected without causing death.

Next, in order to test the development of immunity to ricin, the animals were bled at the end of 14 days. The blood was always taken from the ear vein. A few days after the sample of blood had been taken, the animals were each injected subcutaneously with a lethal dose of untreated ricin. All of the rabbits lived.

The sera of the rabbits were tested for their antiricin properties. Various quantities of the sera were mixed with lethal doses (for adult white mice) of ricin and injected subcutaneously into adult white mice. The sera from the rabbits neutralized a lethal dose of ricin when used in as small quantities as 0.1 cc. except that from rabbit No. 54, which required 0.2 cc. of serum to detoxify a lethal dose.

CONCLUSIONS.

1. Sodium ricinoleate detoxified ricin.
2. An immunity to ricin is developed by means of one injection of a mixture of ricin and sodium ricinoleate.
3. The sera from the animals treated as above have antiricin properties, and even in as small quantities as 0.1 to 0.2 cc. detoxify a lethal dose (for adult white mice) of ricin.

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[1] Larson, W. P., and Montank, I. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 229; Larson, W. P., and Nelson, E., *ibid.*, 1924, xxi, 278; Larson, W. P., Evans, R. D., and Nelson, E., *ibid.*, 1924, xxii, 194; Larson, W. P., and Nelson, E., *ibid.*, 1925, xxii, 357; Larson, W. P., and Colby, W., *ibid.*, 1925, xxii, 549; Larson, W. P., and Halvorsen, H. D., *ibid.*, 1925, xxii, 550; Larson, W. P., Hancock, E. W., and Eder, H., *ibid.*, 1925, xxii, 552.

[2] (To be published soon.)

[3] Osborne, T. B., Mendel, L. B., and Harris, I. F., *Am. J. Physiol.*, 1905, xiv, 259.

3208

Disintegrative Action of KNC on Amblystoma Embryos in Solutions of Different Osmotic Pressures.

J. WILLIAM BUCHANAN. (Introduced by George A. Baitsell.)

From the Osborn Zoological Laboratory, Yale University, New Haven, Conn.

A series of experiments were performed dealing with the effect of varying the osmotic pressure of the solution, on the disintegrative action of potassium cyanide, on the early embryos of *Amblystoma punctatum.* The experiment was carried on during the Spring of 1926. Owing to the unusually short season, the work is incomplete in many respects, and further experiments are planned for future seasons. However, certain very definite results have been obtained, which appear to have an important bearing on the general problem of susceptibility and susceptibility gradients.

In each experiment a batch of embryos from a single female was selected and dissected free from the jelly capsules. This was necessary because preliminary experiments showed that the capsules afford some protection against KNC. The embryos were allowed to remain for a number of hours in cold running tap water, during which time any that had been injured died. To freshly made solutions of commercial cane sugar, Ringer's solution, urea (made up in both distilled and tap water), and to distilled and tap water was added sufficient KNC to make the desired concentration. The range of concentrations employed was: Sugar, 0.03 molal to 0.6 molal; Ringer's solution, 1/10th to double the usual concentration; urea, 0.03 molal to 0.6 molal; KNC M/250 to M/125. As regards the osmotic pressure of the solutions, a crude cyroscopic method served to demonstrate that solutions of sugar of the order of 0.15 molal and Ringer's solution diluted one-half are both hypertonic for the Amblystoma embryo in the stages employed. Saturation with oxygen was assured by shaking the solutions with air. The embryos were then placed in 250 cc. Stender dishes in tap water, and the water replaced by 250 cc. of the desired solutions. The lids of the dishes were sealed with vaseline. Examination of the condition of the embryos was made at suitable intervals, and a record was made of all that showed any indications of death or disintegration. Depending on the number of embryos in the batch, groups of ten or twenty were placed in each dish. The stages used were blastulae and gastrulae. No attempt was made to bring the solutions to neutrality, hence, the results are the sum of the specific effect of the reagents and that of the alkalinity of the solutions. The distilled water used was once distilled in a copper still equipped with a block tin condenser.

From the data obtained in 15 experiments thus far completed, the following facts are clear: (1) Embryos die more rapidly in distilled water solutions of KNC than in like concentrations made up in tap water. The difference is equally evident between distilled and tap water solutions of urea, hypertonic sugar, and hypertonic Ringer's, to which KNC has been added. The additive action of KNC and distilled water of the sort employed here is, therefore, plainly not due to the hypotonicity of the KNC-distilled water solutions.

(2) In general, embryos disintegrate more rapidly in solutions of cyanide made up in Ringer's solution in at least two concentrations (full strength and one-half strength) than in such solutions made up in tap water. This additive action of KNC and Ringer's solution makes it necessary to observe caution in drawing conclusions regarding the rôle of osmotic pressure in the disintegrative action of KNC from results obtained with Ringer's-cyanide solutions.

(3) Urea is toxic in solutions iso-molal with non-toxic hypertonic concentrations of sugar. Conclusions regarding the effect of hypertonic solutions on development as compared with the effect of iso-molal solutions of urea are, therefore, of limited value.

(4) Cane sugar exerts an antagonism toward the disintegrative action of KNC. The protection afforded by the sugar increases with increasing concentration of the sugar up to 0.3 molal. There were no exceptions observed. The results indicate that with increasing concentration of the cyanide, the concentration of sugar necessary to afford greatest protection increases.

Embryos which were intact in strong sugar-cyanide solutions at the end of 48 and 72 hours were transferred to the same concentration of sugar without the cyanide, and the concentration of sugar decreased gradually by the addition of tap water. Some of the embryos disintegrated very soon, others developed slowly for three or four days and then died. From this it may be concluded that while sugar protects the embryo against the disintegrative action of the cyanide, nevertheless the injury is profound and complete recovery does not occur after the exposures employed here.

Indications of death of the embryos differ among the solutions used. In one-half Ringer's solution plus M/250 KNC death in blastulae begins as a gray cap at the animal pole. This cap increases in area and spreads downward and eventually involves the entire surface. Blastulae do not usually burst during the first 72 hours of exposure. The process is similar but much more rapid in 0.15 molal urea plus KNC, and in tap and distilled water solutions of KNC, and is frequently accompanied by the bursting of the blastulae in the vegetal hemisphere. In the case of gastrulae, the region of the closing blastopore is first affected in these solutions. Visible changes are the whitening of the cells about the blastopore, particularly the dorsal lip, followed by the spreading of the whitened area anteriorly involving the neural plate. The neural plate may persist for some time as an area of dead tissue before other regions of the embryo are visibly affected.

Deaths occurring in hypertonic sugar-KNC solutions do not show this differential susceptibility of the animal pole, blastoporic lips, and neural plate. As stated above, death and disintegration in hypertonic sugar-KNC solutions is greatly delayed as compared with the rapidity of these processes in KNC added to the other agents employed. When disintegration finally does occur, no differences in the appearance of the animal pole of blastulae, nor the lips of the blastopore and neural plate of gastrulae, and other regions of the embryo are discernible. There were no exceptions to this among the more than 50 embryos dying in sugar-KNC solutions that were observed. The entire embryo becomes a lighter color and eventually bursts in the region of the yolk cells. In 0.6 molal sugar plus KNC whitened ridges appear, probably due to shrinkage in this concentration of sugar. The processes of disintegration begin at these ridges and soon spread and involve the entire embryo.

Two main conclusions may be drawn from the results of the experiments with sugar-cyanide solutions; first, as regards the disintegration of Amblystoma embryos, KNC and hypertonic sugar solutions are antagonistic. This fact is in line with the well known experiments of Loeb, who employed KNC to prevent the disintegrative action of hypertonic sea water on sea urchin eggs. Second, hypertonic sugar solutions obliterate the differential susceptibility of the several regions of the embryo to cyanide disintegration. Observations on a possible similar action of strongly hypertonic Ringer's solution are incomplete.

3209

Effect on Blood Sugar of Direct Irradiation of Blood in Vivo.*

C. I. REED, J. I. PAYTE AND ROBERT W. LACKEY.

From the Department of Physiology, Baylor University Medical School, Dallas, Texas.

In a paper previously published[1] one of us described a method of irradiating the blood of etherized dogs by allowing a beam from a special carbon arc to fall on a quartz tube inserted in the carotid artery. By this method a series of studies has been undertaken on the effects of this procedure on blood pressure,[1] on leucocytes,[2] on electrophoretic potential of erythrocytes,[3] on blood uric acid,[4] blood calcium,[5] and CO_2 combining power.[6]

It is the purpose of this paper to report the results of this procedure on blood sugar, for the sake of a more complete picture of the effects of such a method of irradiation.

A control series was first run in which the dog was subject to ether anesthesia together with all the operative technic but without irradiation. The first sample of blood was drawn from the saphenous vein before anesthesia. Subsequent samples were drawn from the femoral vein at various intervals. All blood samples were heparinized either *in vitro* or *in vivo*. Determinations were made by the method of Tolin and Wu.[7] The results of the control experiments are shown in Table I.

Another series was made in which irradiation was begun after

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* Part of the expenses of this investigation were borne by a grant from the Research Committee of Phi Rho Sigma Medical Fraternity. Part of the heparin was supplied by Hynson, Westcott and Dunning. The carbon arc lamp was loaned for research purposes by Paul E. Johnson Co.

TABLE I.

Blood sugar, mg. per 100 cc. Time from 1″ blood sample. Ether anesthesia.

Minutes		3	5	10	15	20	25	30	35	40	45	50	55	60	70	80	85	90	95	100	105	110	120	150
1	—							138						154				160					190	210
2	87				95							143				154						154		
3	84			105						228					266					236				
4	60							146						139							153			
5	37			40								100				83						90		
6	93		93					102						113				122						
7	96		107	136		187				230								230						
8	—		63	60	55		60						60						60					
9	87	100		125		133			174			154				180								
10	85		100	125						150					222	235	222	210						
11	74		99		111			148						130				143			181		166	
12	74	76			79						80				80	90	88	95						
13	73					125						166			200			214		230		230	230	

TABLE II.

Blood sugar, mg. per 100 cc. ether anesthesia. Irradiation begun at *.

Minutes		5	10	15	20	25	30	35	40	45	50	55	60	80	85	90	95	100	105	110	115	120	125	130	135
1	60					166			*			200			222				285		285		285	285	
2	83		108					*			160			181			266		296						
3	75			*	143			133			136			187						214		230			
4	120	133					*				147			125						143			166		230
5	108			104					*		102			108						105			108		111
6	130			142						*			166			171						150			130
7	117						130*						136				150			158			—		
8	120		130					*	150									187			230				
9	115					122*							120			115			125	120	111	115			
10	97			142	*			166					120	142		131				136					
11	125				176*					230				250		250			272						

the second sample was drawn. The results of this series are shown in Table II.

Anesthesia usually produced a progressive rise in blood sugar values. Initial values in the two series ranged between 37 and 130 mg. per cc. In two instances (No. 8, Table I, No. 5, Table II) when the animals had not been fed for two days, the level remained practically constant throughout a period of 95 minutes of anesthesia. In all other cases the figures were distinctly higher within 5 to 10 minutes.

The initial figures are generally higher in the experimental series, for which no explanation is attempted, as the animals were selected at random from stock. Curves constructed from these tables, but not included in the paper, are so nearly identical that it can only be concluded in the basis of these experiments that irradiation with a special carbon arc emitting a high percentage of ultra-violet rays has no influence on blood sugar of etherized dogs.

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[1] Reed, C. I., *Am. J. Physiol.*, 1925, lxxiv, 518.

[2] Reed, C. I., *ibid.*, 1925, lxxiv, 525.

[3] Falk, I. S., and Reed, C. I., *ibid.*, 1926, lxxv, 616.

[4] Koch, F. C., and Reed, C. I., *ibid.*, 1926, lxxv, 351.

[5] Reed, C. I., and Tweedy, W. R., *ibid*, 1926, lxxvi, 54.

[6] Moran, W. H., and Reed, C. I., *ibid.*, (submitted for publication).

[7] Tolin and Wu, *J. Biol. Chem.*, 1920, xli, 367.

3210

The Bacteriophagic Relationships Between B. Coli, S. Fecalis and S. Lacticus.

PHILIP HADLEY AND EUGENIA DABNEY.

From the Department of Bacteriology, University of Michigan, Ann Arbor, Mich.

The systematic relationships existing between *S. fecalis* and recognized streptococci of the hemolytic and viridans group, or with acid-forming organisms common to the intestinal tract, such as *B. coli, S. lacticus (B. acidi lactici), M. ovalis* or the "Enterococcus" of Thiercelin and others, have long been a subject of controversy. Andrews and Horder[1] attempted to establish the fecal streptococcus as one of their six streptococcus types. Holman[2] placed it among his various types of streptococcus. Brown,[3] in his comprehensive review of the blood-reactions of the streptococci, states: "Furthermore, strains of *S. fecalis* are green producers and therefore belong to the *S. viridans* group as originally proposed by Schottmüller." Gordon[4] established, on a basis of serological tests, three types—*S. pyogenes* (hemolytic), *S. salivarius* (viridans), and *S. fecalis* ("Enterococcus"). These he also distinguished on the basis of hemolysis, raffinose-fermentation and mannite-fermentation. He also regarded these groups as serologically distinct. Dible,[5] largely on the basis of fermentation tests, attempted to show that there existed an identity between *S. fecalis* and the Enterococcus of Thiercelin, often mentioned by French writers. Kendall,[6] in 1924, placed *S. fecalis* with the greening and hemolytic streptococci in tabular form, but avoided in the text a discussion of its actual position. He mentioned *M. ovalis* as probably identical with the Enterococcus but did not relate it to *S. fecalis*. Ayers and Johnston[7] stated that *S. fecalis* was identical with, or at least similar to, *S. lacticus*. They used as criteria (1) heat-resistance, (2) morphology (oval form), (3) cell-grouping (short chain) and (4) mannite-fermentation. Thus we have some diversity of view on the position of *S. fecalis*.

Without here taking occasion to enter into the consideration of merits or demerits of these various conceptions regarding the actual affiliation of *S. fecalis*, we wish to report briefly the results of certain experiments which seek to attack the problem of the position of this organism from a somewhat different point of view, namely, the bacteriophagic inter-relationships of some of these bacterial species. More explicitly, we desire to place on record the results of some cross-tests involving the use of the bacteriophage or lytic principle, active against *S. fecalis, B. coli, S. lacticus* and, incidentally, *B. typhosus*. It is, however, not within the scope of this paper to consider in any way the nature of the lytic agent or the mechanism of bacteriophagic action.

Quite recently the junior author and E. M. Brill isolated independently a bacteriophage active against a typical S type strain (No. 77, Dental College Collections) of *S. fecalis* obtained about one year ago by Rickert and Hadley[8] from the pulp chamber of an infected tooth. It agreed in morphological, fermentative and serological characters with the typical fecal *S. fecalis,* probably the most common agent in root-canal infections. In addition, a fresh lytic principle was isolated for S type *B. coli* (Jordan strain). These two lytic agents, having been enhanced to a high, but probably not maximum, titer against their respective homologous substrata, were employed in preliminary cross-tests against the homologous and heterologous cultures, and the results several times confirmed. The method was as follows:

Six tubes filled with 10 cc. of sterile beef infusion broth (pH 7.8) were set up, containing

1. One lp. fecalis culture plus 5 gtts. fecalis lytic filtrate.
2. One lp. coli culture plus 5 gtts. fecalis lytic filtrate.
3. One lp. fecalis culture plus 5 gtts. coli lytic filtrate.
4. One lp. coli culture plus 5 gtts. coli lytic filtrate.
5. One lp. fecalis culture ------------------
6. One lp. coli culture ------------------

All tubes were incubated 12 hours at 37° C. and examined for inhibition of growth. Tubes 1, 2 and 4 were perfectly clear. Tubes 3, 5 and 6 showed strong (normal) clouding. The appearance of the tubes was essentially the same, except for further clouding of the control tubes, after 96 hours at room temperature. Thus, the fecalis lytic filtrate gave complete inhibition against *B. coli* as well as against the homologous substratum. The coli lytic filtrate, on the other hand, while completely inhibiting the homologous culture, had no influence on *S. fecalis.* These tests were repeated several times with similar results. The employment of one drop of lytic filtrate instead of five drops gave approximately the same effect. Indeed, a dilution of 10^{-4} of the fecalis lytic filtrate yielded complete inhibition of *B. coli,* while a dilution of 1:1,000 gave complete inhibition of *S. lacticus.*

In view of these findings it seemed desirable to ascertain the bacteriophagic relationship of *S. lacticus* to *S. fecalis* and *B. coli.* Accordingly a bacteriophage active on an old laboratory strain of *S. lacticus* was produced and the following tests performed. At the same time a comparison was made with the action of the fecalis lytic principle.

From Table 1 it is apparent that lytic filtrates active against both *S. lacticus* and *S. fecalis* give a complete inhibition of growth of *B. coli* as well as of the respective homologous culture. How-

TABLE I.

Showing action of lytic principle for *S. fecalis* and a lytic principle for *S. lacticus* against *B. coli*, *S. fecalis* and *S. lacticus*. (Results in terms of inhibition.)

Substratum: One loop of culture in 10 cc. broth.	Amount of L. P. Lacticus		Amount of L. P. Fecalis		Culture control
	5 gtts*	1 gtt**	5 gtts	1 gtt	
B. coli	C	C	C	C	—
S. fecalis	—	—	C	C	—
S. lacticus	C	C	C	2+	—

*Dilution of L. P. calculated at 1:50; ** as 1:250.

(C) complete inhibition of growth for 24 hours or more.

(3+), (2+), (1+) intermediate grades of inhibition.

(—) no inhibition observed.

ever, while the filtrate active on *S. lacticus* has no action on *S. fecalis*, the fecalis lytic filtrate is effective in inhibiting the growth of *S. lacticus*.

Having now ascertained the existence of a bacteriophagic relationship between *S. fecalis* and *B. coli*, also between *S. lacticus* and *B. coli*, as well as an asymetrical relation between *S. fecalis* and *S. lacticus*, it was of interest to observe the possible relationship between the streptococcus strain and *B. typhosus*. A bacteriophage for an old laboratory strain of the S type (Eberth) was therefore produced and used in the tests described in the following table, along with a repetition of the former tests. The method was the same as previously described.

TABLE II.

Showing the action of lytic principle derived for *B. typhosus*, *B. coli*, *S. lacticus* and *S. fecalis* respectively, against the homologous and heterologous substrata. (Results expressed in terms of inhibition.)

Substratum: One loop of culture in 10 cc. broth.	Added one drop* of L. P. for				Culture control
	Typh.	Coli	Fecalis	Lacticus	
B. typhosus	C	3+	2+	2+	—
B. coli	C	C	C	C	—
S. fecalis	—	—	C	—	—
S. lacticus	—	—	C	C	—

Symbols the same as in the preceding table.

*Dilutions calculated as 1:250.

From the results presented in Table II, which serves as a summarizing statement of all the reactions previously considered, it appears that the fecalis and lacticus lytic filtrates behave in the same manner when brought into action against *B. coli* and *B. typhosus* substrata. Moreover, that the fecalis and lacticus substrata comport themselves in an identical manner toward *B. coli* lytic filtrate and toward *B. typhosus* lytic filtrate.

It may be added that the lytic filtrates in each case were checked for their ability to produce typical lytic areas when streaked, in appropriate dilution, against the homologous substratum. The titer was such that one or more standard loops of (one standard loop of stock lytic filtrate plus 10 cc. of sterile broth) gave countable areas (*i. e.,* a few to 100) after incubation for 24 hours on slanted agar. The maximum observed inhibition-titers in broth for the various filtrates were as follows: *B. coli,* 10^{-7}; *B. typhosus,* 10^{-8}; *S. fecalis,* 10^{-9}; *S. lacticus,* 10^{-7}. That is to say, a 10^{-7} dil. of *B. coli* filtrate in 10 cc. of broth inhibited the growth of one standard loop of young coli broth culture.

We are of the opinion that the evidence suggests, though by no means demonstrates, the affiliation of the fecal streptococcus with members of the colon-typhoid group of bacteria rather than with the true streptococci as represented by the viridans and hemolytic types. Indeed, the bacteriophagic reactions of *S. fecalis* and *S. lacticus* show a considerable degree of uniformity. That the relation is close, however, is by no means necessarily implied, for we know that the reactions of any specific lytic principle often, and perhaps usually transcend the limits of the species and group. This has already been demonstrated by one of us[9] in an instance in which typhoid lytic principle caused lysis of *B. pullorum* and *B. gallinarum,* as well as of the homologous substratum. In this case the reaction was also in large measure reciprocal, and was in harmony with the corresponding serological tests. However, we know of no instance in which a specific lytic agent, already well adapted to a member of the colon-typhoid-dysentery group, has been found to act directly against such distantly related bacterial species as staphylococcus or streptococcus. At the same time we see that, within narrower limits, the bacteriophagic relationships between various species seems to exist on a fairly broad footing; and that these relationships are certainly more comprehensive than those demonstrated by the usual serological tests. Indeed, the bacteriophagic relationships seem to be more in harmony with those serological reactions existing between the R types of bacteria (from active microbic dissociation) represented by Schütze[10] in his so-called "serological cosmopolitanism" of the R forms; also by the commonly recognized serological heterogeneity of the pneumococcus types as indicated by the studies of Stryker,[11] Yoshioka,[12] and, more recently, Reimann[13]; also by the serological heterogeneity of the O and R types of *B. typhosus* and *B. enteritidis* as studied by Goyle.[14] To what extent serological reactions among R type cultures (as opposed to S type cultures, or cultures in any other cyclogenic state) parallel bacteriophagic reactions may prove an interesting study and one that may bear closely upon significant aspects of the problem of transmissible autolysis.*

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* Since this paper went to press the authors have tested the action of fecalis lytic principle against strains of *S. viridans* and hemolytic streptococcus. No inhibition was observed in any case.

3211

Light Transmission Through Blood Suspensions as Recorded by the Photo-electric Cell.

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In an attempt to find a method of securing accurate time curves of red blood corpuscle hemolysis by various hemolytic agents, an apparatus was devised in which a constant beam of light was passed through a dilute blood suspension contained in a revolving glass chamber. The emergent beam was allowed to fall on a potassium hydride photo-electric cell, and the current so set up in the cell was suitably measured.[1] Using, under standard conditions, supposedly unchanging blood suspensions in various isotonic fluids including saline, Brinkman's solution,[2] several modifications of it, a special phosphate solution, and a modified Locke's solution,[3] marked spontaneous variations in the emergent beam were noted during the first one to three hours after the preparation of the suspensions. Usually these variations consisted of a gradual progressive diminution in light intensity; sometimes an increase occurred.

Studies of the pH of the diluting fluids before and after the experiments revealed no relationship to the light transmissibility of the suspensions. Microscopic observations of the cell shape on flamed slides[4, 5] showed various types of crenation but no correlation between changes in cell shape and in light transmission. The cell volumes of the suspensions were determined in a special hematocrit, and in general light transmission varied inversely as total cell volume, but no definite quantitative relation could be determined.

The following technique was eventually developed to obtain a constant red cell suspension. Washed cells to represent 0.5 per cent whole blood were added sterilely to Brinkman's solution containing 0.1 per cent $NaHCO_3$, and allowed to stand 18-24 hours in paraffined flasks. All of 54 such suspensions showed less than 2 per cent change in light transmission during a three hour period. Of 21 control suspensions on which the light transmission was determined 15 minutes after dilution and again in 1 to 3 hours, only 14 showed no change. Similar experiments using isotonic saline showed a change in about one-third, both before and after standing. The cells after standing in the modified Brinkman's solution had assumed a spherical shape with numerous small delicate projections, and no further change in shape was detected during the period between determinations. Presumably they had reached an approximate equilibrium with the surrounding fluid. This method is being utilized at present in studying the rate of hemolysis of the red blood corpuscles by various hemolytic agents.

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* Fellow in Medicine, National Research Council.

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[1] We are gratefully indebted to Dr. H. E. Ives and Mr. H. C. Snook of the Bell Telephone Laboratories, New York, for the photo-electric cell, and to Prof. H. W. Webb and Mr. D. R. White of the Department of Physics, Columbia University, for the electrical hook-up.

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3212

A Comparison of Indifferent Substances and Specific Antigen in Production of Local Streptococcus Immunity.

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Our recent communication[1] which described a high grade of localized antistreptococcus protection in the pleural cavity of the rabbit induced by the previous injection of indifferent, non-antigenic substances, left in question the relation of this protection to a specific immunity which might be produced by the streptococcus. A thickened granulating parietal pleural wall containing large numbers of phagocytic clasmatocytes is produced by the inoculation three days previously of gum arabic broth or aleuronat-starch and such a cavity resists infection by many multiples of the normally fatal dose of our pathogenic streptococcus. The duration of this protection is from ten days to two weeks and its disappearance is paralleled by a return of the pleural wall to approximately its original condition as we have learned from recent histological study.

Repeated injections of the original non-specific substance, or of considerable doses of the streptococcus in the second and subsequent inoculations, produced further thickening of the pleural wall, an increased number of cells, and an apparently still more marked resistance to the streptococcus. In other words, there was an indication that the degree of resistance varies directly with the number of clasmatocytes present in the protected area. We at once raised the question, however, of the significance of specific antibodies, whether localized or general, in conditions of this sort. In other words, will granulation tissue of a given thickness produced by gum arabic broth afford as much protection as an equal accumulation of cells produced by the streptococcus? A preliminary experiment which we published seemed to answer this question in the negative but was not regarded as conclusive, owing to certain technical difficulties involved.

Pleural cavities that have received aleuronat or gum arabic withstand repeated injections of virulent streptococci but, although the animal recovers perfectly and on examination the cavity is found sterile, it is frequently found obliterated by adhesions in whole or in part. In a similar way one of our control series consists of animals with granulating pleural walls produced by *Staphylococcus aureus.* The injection of this organism is usually not fatal but almost invariably leaves localized abscesses behind, from which pure cultures of the inoculated coccus may be obtained. These two conditions make it impossible to locate a test dose of streptococcus in the pleural cavity with any certainty. To obviate these difficulties we have proceeded as follows in our recent experiments.

Three series of rabbits are prepared by intrapleural injections of:

A. Gum arabic broth.

B. Gum arabic broth containing first dead and then living cultures of Streptococcus "H".

C. Gum arabic broth containing living *Staphylococcus aureus.*

Three or four such injections were given at intervals of 4 or 5 days in equal total volumes for each series. Six or 7 days later representatives of each series were tested with increasing multiples of the minimal lethal intrapleural dose administered into the *left* and still unaffected pleural cavity. We are now well assured of the conditions under which crossed pleural immunity is possible as outlined in the preceding article.

The results of these latest experiments are uniform and unmistakable. Repeated injections of gum arabic broth alone protect as well or even better than the same injections including streptococcus or staphylococcus. Thus in one experiment representatives of each of the three series withstood 3,000 lethal doses. but, only the one with gum arabic alone withstood 15,000 doses. One hundred fifty thousand and 300,000 doses were fatal to representatives of each of the series. In another experiment gum arabic animals withstood 1,800 and 4,500 lethal doses, both of which were fatal to the streptococcus-gum arabic and staphylococcus-gum arabic animals.

An objection may still be raised that the conditions of our experiments do not include true specific immunity; we failed to demonstrate agglutinins in the blood serum of any of our animals before the test injection, including those whose treatment included the streptococcus. Agglutinins appeared in some of the animals of each series during their period of recovery from the test injection. At all events these experiments indicate that a high grade of local immunity may be produced by non-specific substances entirely dependent on increase of clasmatocytes. The failure of the addition of the specific antigen to increase this protection seems somehow associated with the generally recognized inefficiency of streptococcus serum therapy.

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[1] Gay, F. P., Clark, A. R., and Linton, R. W., *Arch. Path.*, 1926, i, 857.

3213

Transpleural Mobilization of Clasmatocytes with Coincident Streptococcus Protection.

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In a recent article[1] we have shown that the presence of granulation tissue in the parietal pleura of the rabbit, produced by previous introduction into the pleural cavity of indifferent substances, protects the animal against many fatal doses of a streptococcus that normally causes a fatal pleurisy. The marked phagocytosis of the streptococcus by the clasmatocytes, which are the predominating cells in this granulation tissue, is regarded as responsible for this type of active resistance against our invasive microorganism. In a more general way we should regard the accumulation of these rather mobile and resistant mononuclear cells of the connective tissue, as responsible for at least certain instances of local immunity.

In the latest of our published experiments we have indicated that clasmatocytes accumulated in the parietal wall of one pleural cavity may be transferred to the other pleural cavity and there likewise protect against the streptococcus. A granulating right pleural wall was effected in a routine fashion by injecting aleuronat-starch mixture or gum arabic broth three days previously. The parietal wall of the treated cavity is from five to ten times as thick as usual, but the wall of the opposite cavity remains normal. If one injects multiple fatal doses of the streptococcus into the left untreated cavity, the rabbit, however, recovers perfectly, although sterilization of the cavity is distinctly slower than when the infection takes place on the right treated side. Histological examination at this stage of the two sides shows that the originally treated right pleura is thickened as usual but consists of myxomatous tissue in place of the densely cellular granulation tissue usually met with. The left parietal pleura is only slightly thickened but packed in between the subjacent muscle fibres are dense masses of clasmatocytes which are never normally found in such numbers or position.

The natural conclusion to be drawn from such an experiment is that the clasmatocytes that had accumulated in the granulation tissue of the right pleura were directly transported and remobilized in the left pleura under the stimulation of the local infection. Such a conclusion, however, is based on certain assumptions that it is the object of this brief communication to verify.

Tenderloo in his treatise on General Pathology has considered in some detail the overlapping of lymphatic distribution particularly of the thorax, which would render possible the migration of cells from one pleural cavity to the other; cancer metastasis from one axilla to the other, and the extension of tuberculosis from lymph nodes of one bronchial tree to that of the other side as in the cases of Ghon and Roman, are matters of record. It was more important, however, to demonstrate that in our experiment the clasmatocytes that were subsequently mobilized in the left pleural wall were those that had been collected in the right pleura and not derived from some parent source elsewhere. This we have demonstrated in the following manner: granulation tissue is produced in the right pleura and the clasmatocytes in it are selectively stained by a single injection of trypan blue. It may be shown in controls that the dye administered in this fashion stains only the locally accumulated cells in the area through which drainage takes place; the scattered cells of the visceral pleura of the same cavity, for example, are not stained.

At all events subsequent injection of aleuronat or broth in the left pleura mobilizes stained clasmatocytes in the left pleural wall, and all such cells disappear from the granulation tissue of the right pleural wall. One cannot use the streptococcus successfully in such an experiment because although the stained cells mobilize on the left side, a fatal infection, which clouds the picture, follows. This unusual result can in turn be explained by the plugging of the clasmatocytes with dye which renders them unable to fulfill their protective phagocytic function. Various other controls have been introduced as, for example, dye injection into a normal right cavity, followed by failure to find mobilized cells on the left side, and lack of protection.

These additional experiments confirm and extend our belief that the clasmatocytes which may be accumulated in a given region are responsible for a high grade of local protection against the streptococcus. Furthermore, a collection of clasmatocytes in one region may be transferred to adjacent and possibly to more remote regions and still exercise a protective function.

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[1] Gay, F. P., Clark, A. R., and Linton, R. W., *Arch. Path.*, 1926, i, 857.

3214

Non-Specific Activation of the Alcohol Soluble Antigen of Tubercle Bacillus.

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In a previous publication[1] it was found that the addition of commercial lecithin and alcoholic heart extract increased the potency of an alcohol extract of the tubercle bacillus as an antigen in the complement fixation reaction about ten times. The potency of certain preparations derived from the alcohol extract was increased about 100 to 200 times. Further work on this subject, although it is not yet completed, revealed the following characteristics.

An alcohol or ether extract of the tubercle bacillus and the preparations representing different stages of the purification of them, as described in our first paper[2] are activated with the addition of lecithin only if they are mixed with the lecithin in alcoholic solution and diluted together. The addition of saline suspension of lecithin to the saline suspension of the specific substance has no activating effect. Contrary to this, the preparations described later in these Proceedings[3] are activated whether the lecithin is added to the alcohol or to the saline solution of the preparations. The different behavior of the two classes of preparations toward lecithin is due, probably, to the circumstance that the substances later mentioned contain the specific substance nearly or entirely separated from the lipoids and are water soluble. In the experiments described in the first publication we examined in saline solution only the substances belonging to the second group showing strong activation, and we did not observe that the others are not activated in saline suspension.

With the bacterial substances of the first class, which are activated only if mixed in alcohol solution with lecithin further work was done in two directions. The effect of an increasing amount of lecithin was studied, and a marked optimum was found. The potency was increased about sixteen times with 0.004 to 0.002 mgm., about 4 to 8 times with 0.009 mgm., and only twice with 0.0005 mgm. With 0.00025 mgm. no activation was present. Then the effect of different substances other than lecithin was studied, namely, petrolatum jelly, hog fat, stearic and oleic acid, the mixture of the acids in different proportions with the hog fat and petrolatum, and benzoin of sumatra.[4] The fatty acids were examined because our lecithin preparations contained a considerable amount of free acids, and the benzoin because it favors, according to several authors, the specific precipitation of alcoholic bacterium extracts. The cholesterin had previously been studied. Only the petrolatum jelly has shown a slight activation (four times). The admixture of the others in different proportion to the specific substance was without any effect. Only further work can decide whether the activating effect of commercial lecithin and alcoholic organ extracts is due to the phosphatids contained in them or to some concomitant substance, and whether the mechanism is mainly physical or chemical. That the addition of different substances to the alcohol solution of the specific substance, which influence largely the properties of the saline suspensions, and which has had practically no effect on the activity of the antigen, is in contrast to the usual conception that the potency of the alcohol soluble antigens for the complement fixation reaction is influenced to a great extent by the physical properties of their suspension. In the case of the alcohol soluble antigen of the tubercle bacillus no evidence was found for such an influence.

The activation of the preparations belonging to the second group, which are activated also by a saline suspension of lecithin, has not yet been further studied.

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3215

The Presence of Two Different Antigens in Alcohol Extract of the Tubercle Bacilli.

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The first thorough investigator of this subject, Meyer,[1] supposed that in the lipoid extract of the tubercle bacillus two different antigens are present and are characterized, beside serological differences, by their different solubility in ether and alcohol. The slight serological differences described by Meyer did not seem to one of us to be sufficient ground for this supposition, all the more as the solubility of the specific substance was found different from the solubility of the preparations of Meyer.[2] Our present observations show that considerable serological differences can be found between certain fractions of the extract, although we are not able to ascertain the true significance of these differences. As was found by Meyer, the serological differences are connected with different solubility of the preparations.

A methyl alcohol extract of the tubercle bacillus strain 597, obtained after previous acetone, ether, and ethyl alcohol extraction, was evaporated and dissolved in ether. The ether soluble part was precipitated twice with four volumes of acetone at 37° C. The acetone precipitate was extracted three times with large amounts of ethyl alcohol under thorough grinding of the precipitate with a glass rod. The alcohol insoluble part was dissolved in ether. When the smallest amount of the alcohol and ether soluble substance giving positive complement fixation reaction was determined with two tubercle bacillus sera, one obtained with the fast growing strain 597, the other with the freshly cultivated strain R, from rabbits, considerable difference was found between the two preparations. The ether solution reacted with one fourth the amount of serum R than with serum 597. The alcohol soluble substance reacted in two and a half times the amount with serum 597 than with serum R. The figures for the alcohol soluble substance in the table were obtained when the potency of the preparation was considerably diminished. The same difference is systematically found with the preparations obtained in the way described, and with the derivatives of them, not only with the tubercle bacillus, but in the case of a smegma bacillus strain examined, and with different sera obtained with the same strains.

When the ether solutions of the two substances described are shaken with distilled water, and centrifuged, three layers are formed; an ether solution, an inseparable jelly (formed of ether solution and water) and water solution. When the shaking with distilled water is repeated several times all the specific substance and all the dissolved lipoids are taken out from the ether solution, the largest part of them being found in the jelly. From a non-purified extract, the specific substance is taken out in this way from an ether solution, the non-specific lipoids remaining in the ether. Treating the alcohol non-soluble fraction of the methyl alcohol extract (in ether solution as described) with water, the specific substance is found nearly undiminished in the jelly and in the water solution, both reacting better, before water treatment, with serum R than with serum 597. The specific substance soluble in alcohol is in large measure destroyed by the described procedure, and it can be found only in the jelly. The water solution, although it contains considerable amounts of organic substance, has little or no antigenic effect.

The water solution, obtained in the described way from the alcohol non-soluble part of the methyl alcohol extract, shows usually in higher concentration an inhibition of the specific reaction. In one instance with 0.025 to 0.001 cc. the reaction was negative. It was positive only with .0005 to .0001 cc. The alcohol soluble antigen of the tubercle bacillus usually shows no inhibition even when high concentration, 500 to 1,000 antigenic units, can be examined. The addition of the watery solution described to other antigens not showing inhibition of the reaction in higher concentration, inhibits to a great extent the complement fixation with the serum R, and does not inhibit at all with the serum 597.

With the absorption experiment we were not able to differentiate the two antigens. The suspension of one, absorbed the antibodies also of the other. But it might be that the antigens in the preparation used were not sufficiently separated from each other.

The observations, except the absorption experiment, favor the conception that the antigen, non-soluble in alcohol and reacting better with serum R, is different from the alcohol soluble substance reacting better with serum 597. But there is an observation which appears to be in contrast with this supposition.

The preparation, described in a former note in these PROCEEDINGS,[3] which is probably separated from the lipoids, reacts better with serum 597 than with serum R, although so far it has been obtained only from preparations corresponding to the alcohol non-soluble substance reacting better with serum R. It is not yet decided whether or not it is the derivative of this substance or of the alcohol soluble substance contaminating it.

We have not yet studied the individual behavior of numerous sera. But the following observation might be of use for the interpretation of the difference found between different fractions of the lipoid extract: Four rabbit sera obtained with the strain 597, which we used extensively, gave indistinguishable reactions with the different preparations. But the sera, immediately after they are taken from the rabbit, give positive reactions with 5 to 10 times smaller amounts of the preparations than when examined a few weeks later. Not the antibody titer of the sera, but the avidity of the antibodies, diminishing rapidly in the first weeks, decides with how small an amount of antigen we will obtain positive reaction. It might be that the cause of the differences described in this paper between the different fractions of the extract is that the avidity of the antibodies is different in different sera toward the same specific antigen in different chemical binding.

TABLE I.

	Antigen Unit with Serum R	Antigen Unit with Serum 597
597 tubercle bacillus methylalcoholic extract, alcohol non-soluble fraction	.00018 mgm.	.0007 mgm.
The wash water of the ether solution of the former	.00025 mgm.	.0006 mgm.
597 tubercle bacillus methylalcoholic extract, alcohol soluble fraction	.0018 mgm.	.0008 mgm.
The wash water of the ether solution of the former	.06* mgm.	.06* mgm.
The specific substance probably separated from the lipoids, alcohol solution	.06 cc.	.008 cc.
Smegma bacillus methylalcoholic extract, alcohol non-soluble fraction	.00065 mgm.	.0005 mgm.
Smegma bacillus methylalcoholic extract, alcohol soluble fraction	.0015 mgm.	.0001 mgm.

* No reaction.

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[2] Dienes, L., and Schoenheit, E. W., *J. Immunol.*, 1925, x, 631.

[3] Dienes, L., and Schoenheit, E. W., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 106.

3216

Alcohol Soluble Specific Substances of B. Diphtheriae and of Streptothrix.

J. FREUND. (Introduced by L. Dienes.)

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The alcohol soluble specific substance of *B. diphtheriae* was studied, and an effort made to demonstrate such antigens in different micro-organisms, especially organisms related to *B. tuberculosis* and *B. diphtheriae,* and higher pathogenic fungi. The organisms were grown on 0.2 per cent dextrose broth, washed, dried, extracted twice with acetone and twice with alcohol. The immune sera were obtained from rabbits injected with suspensions of the micro-organisms. The titer of the diphtheria immune serum I and II in the complement fixation was 1:160 with the alcoholic extract, 1:160 with the bacillary suspension, and 1:160 with diphtheria broth. The titer with the alcoholic extract dropped in the course of one year to 1:40 and 1:15. The titer of the streptothrix serum I was 1:40 and that of II, 1:10 with alcoholic extracts, while with suspension of streptothrix serum II reacted stronger (1:300) than serum I (1:80). In a few months the titer of serum I dropped 1:15. No alcohol soluble antigens could be demonstrated in the case of *B. xerosis, trichophyton* and yeast, although the sera reacted with the suspensions of the respective organisms. With two strains of *actinomyces* no sera giving complement fixation were obtained. The negative results do not exclude the possibility that other, perhaps freshly isolated strains, would incite antibody production against the alcohol extracts.

The alcohol extract of *B. diphtheriae* gave positive complement fixation neither with tubercle bacillus nor with streptothrix immune sera. The diphtheria immune serum reacted in a dilution 1:10 with suspension of tubercle bacillus and in a dilution 1:30 with alcoholic extract of the same, but reacted neither with the suspension nor with the alcoholic extract of streptothrix.

In the Castellani experiment, employing filtration through paper pulp, the alcoholic extract absorbed the antibodies for itself and for the bacillary suspension. Since it is quite probable that the bacillary suspension contains other antigens than those present in alcoholic extract it is quite possible that the antibodies corresponding to the different antigens are combined with each other. Potent sera suitable for the Castellani experiment have not yet been obtained by the injection of watery extract free from alcohol soluble antigens.

Dilutions of the alcoholic extract 1:10 and 1:100 gave precipitation with the undiluted immune sera in the ring test. Diphtheria toxin (received through the courtesy of Dr. C. Shore, Laboratory of Hygiene of North Carolina) gave no positive complement fixation with our immune sera, nor did an antitoxic serum with the alcohol extracts, although the broth from which the growth was removed by centrifugalization, without appreciable toxicity, was more potent antigen than the bacillary suspension or the alcoholic extract.

The alcohol soluble antigen is readily soluble in methyl alcohol, in acetone and in weak alkali; insoluble in ether, in weak acids, and in water. The potency of an alcoholic extract, measured by the smallest amount of dry material giving complement fixation (2 units of complement in the volume of 0.6 cc.) was increased by the following procedure of purification: The acetone or alcohol extract was evaporated *in vacuo* at 45° C., extracted with ether, dissolved in methyl alcohol, precipitated by HCl, dissolved in weak Na_2CO_3 or in NaOH, the precipitation repeated twice, the final precipitate dissolved in methyl alcohol or in weak NaOH. The potency of the different purified preparations is quite uniform.

	Unit for the complement fixation test	P. Content	N Content
	mgm.	Per cent	Per cent
1. Acetone extract	0.001	0.15	
2. Purified preparation from 1	0.0004		
3. First alcoholic extract	0.0030		
4. Purified preparation made from 3	0.00045	0.68	6.7
5. Second alcoholic extract	0.0016	0.42	4.8
6. Purified preparation made from 5	0.00034		5.6
7. Purified preparation made from 5, dissolved in alkali	0.00046	0.5	

In two purified preparations, prepared from the second alcoholic extract, after boiling with $Ba(OH)_2$, no ether soluble substance (fatty acids) could be found, nor after treatment with 25 per cent H_2SO_4, could reducing substances be demonstrated. Pepsin and trypsin did not decrease the potency of a purified preparation. The addition of lecithin to the first alcoholic extract, when the mixture contains 99 per cent of lecithin expressed in dry material, enhances the potency four times.

The examination of the extract of streptothrix has been retarded because of the lack of a highly potent serum, and because of the rapid drop of potency of the serum available.

The smallest amount of the alcoholic extract giving complement fixation was 0.006 mgm. The activation of this extract by the addition of lecithin is very striking. The antigen and lecithin were mixed in alcoholic solution. In a mixture containing 0.2 per cent of the alcoholic extract an activation 200 times was observed.

3217

Local Hypersensitiveness in Tuberculous Guinea Pigs.

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The plan of the experiments described in this note was suggested to us by the papers of Lewis and Loomis[1] on allergic irritability.

Guinea pigs shortly after a tuberculous infection, during the interval when the tuberculin sensitiveness usually appears, were treated with egg white, and later the skin sensitiveness toward egg white and the appearance of precipitins and complement fixating antibodies in the blood serum were examined. Non-tuberculous guinea pigs were treated and observed in the same way.

Table I contains the observations with one series of animals. The other series, together with the detailed description of the observations, will be published later.

Six control guinea pigs were treated together with the series described in the table; three were treated like the guinea pigs 1-3

TABLE I.

No. of guinea pig	Treatment of the guinea pig	Skin reaction on the 8th day after treatment; 0.1 mgm. egg white (dry weight) intracutaneously. Reaction after			Precipitation	
		6 hours	24 hours	48 hours	9 days after the treatment	13 days after the treatment
1	Guinea pigs 1-3 The 8th, 11th and 13th day after the infection, 0.1, 0.5 and 1.5 mgm. egg white (dry weight)	20x20 lsw.	25x25 lsw.	no reaction	10000++ 100000±	300000++ 1 million±
2		ssw.	25x25 sw. 10x10 bn.	17x17 sw. 9x9 n.	100++ 10000±	1 million++
3		28x28 lsw.	30x30 lsw.	no reaction	10— 1000—	1000±
4	Guinea pigs 4-6, in the same days as former, 0.03, 0.15 and 0.5 mgm. egg white.	20x20 sw.	25x25 lsw. 6x6 bn.	15x15 lsw. 5x5 n.	1000++ 10000±	300000++ 1 million±
5		18x18 ssw.	25x25 lsw. 9x9 bn.	20x20 sw. 10x10 n.	1000++ 10000±	1 million++
6		no sw.	20x20 sw. 8x5 bn.	14x14 sw. 6x8 n.	100— 10000—	10000± 300000±
7	Guinea pigs 7-9, in the same days as former, 0.01, 0.03 and 0.1 mgm. egg white.	18x18 ssw.	30x25 lsw.	20x20 lsw. 10x10 bn.	1000++ 10000±	300000++ 1 million±
8		20x20 sw.	20x20 ssw.	no reaction	100± 1000±	100± 10000±
9		25x25 sw.	30x30 lsw. 11x11 bn.	25x25 lsw. 12x12 n.	1000++ 10000±	1 million++

With the skin reaction, the numbers signify mm. sw. = swelling; lsw. = large swelling; ssw., = slight swelling; n = necrosis; bn. = beginning necrosis. With the precipitation, the numbers signify the highest dilution of native egg white giving a strong (++) or a slight (±) reaction with the dilution 1:2 of the serum.

of the table, three with larger doses. Only one of these guinea pigs, treated with the larger amounts of egg white, has shown a small reaction, fading in 24 hours, in the eighth day after treatment. The same guinea pig gave a faint precipitation with the dilution of egg white from 1:10 to 1:5000. After more prolonged treatment, we found in correspondence with former observations, a quite extensive wheal, which disappeared in 36 hours, after intracutaneous injection of egg white. At the same time, we found precipitation with higher dilutions of the egg white (in one serum 1:10,000, in another 1:100,000), but with all dilutions the precipitation appeared only later, and remained faint compared with the precipitation given by the serum of the tuberculous guinea pigs. Even after prolonged treatment we found no complement fixation with the serum.

It is at once visible from the table that, corresponding to the observations of Lewis, there is a very great difference in the precipitin formation between the normal and infected animals. The same difference is present concerning the antibodies responsible for the complement fixation. The serum of several infected guinea pigs has shown a titer of 1:250 or 1:160 in the complement fixation test. As these antibodies appear only later in the serum, they are not included in the table. In the tuberculous animals, treated even with the smallest dose of egg white (0.14 mgm.) and very soon after treatment, severe necrotic skin reactions were observed.

Two types of severe local hypersensitiveness, leading in the tests to tissue destruction, are known: the Arthus phenomenon, mainly observed in the rabbit, and the tuberculin reaction and similar reactions found in certain chronic diseases. It is undecided whether these two types of local sensitiveness are essentially different, or, though they differ considerably in appearance, the underlying mechanism for both is the same. To which of these two types does the sensitiveness observed by us belong? Is it an Arthus phenomenon, the observation of which was made possible in the guinea pig by the great increase of the antibody production caused by the tuberculous infection? Although a thorough discussion of this question has to be postponed until more experience is gained, some observations are worth mentioning now.

The connection between the antibody content of the serum and the local sensitiveness, although a general parallelism is present, is not so close that the serum antibodies could be held responsible for the sensitiveness. For instance, animals 1 and 6 of the table are exceptions to the rule. The sensitiveness appears earlier than the serum antibodies. The large increase of the antibodies from the 9th to the 13th day after treatment described in the table, was not followed by a corresponding increase of sensitiveness. All experiments for transferring the sensitiveness with blood serum to tuberculous and non-tuberculous animals, remained unsuccessful. We tried to transfer by three methods: the injection of larger amounts of serum (6 and 3 cc.) intracardially; the technic used for the testing of atopic reagins; and the injection of the mixture of serum and egg white in the skin.

The amount of egg white needed both for the sensitizing, and for the production of skin reaction, is very small. There is no indication in our table that the smallest amount used by us (0.14 mgm.) represents the smallest sensitizing dose. With somewhat larger amounts of egg white (13 mgm.) we obtained markedly less sensitization than with the smaller doses. Two hundredths mgm. egg white caused necrotic skin reaction in several animals, and .004 mgm. a marked reaction. Usually in a tuberculin sensitive animal and the protein substances of the tubercle bacillus, the corresponding doses are about .005 and .001 mgm.

The development and appearance of the reactions show considerable variation. In certain cases, in a few hours after the injection of egg white, a 20 to 30 mm. well circumscribed red wheal forms with considerable swelling. In some cases the redness and swelling increases until the next morning; then in 12 hours disappears. (Guinea pigs 1, 3, 8 and all the non-infected controls, after energetic treatment with egg white, reacted in this manner.) In other cases showing an early reaction, a hard swelling and necrosis develops later. But some of the strongest reactions appeared during the night and have shown the appearance and characteristics of the intracutaneous tuberculin reaction, with perhaps the one difference that the necrosis developed earlier than in the tuberculin reaction. Without having more experience, and without examining different antigens, it is impossible to evaluate the significance of the similarities and differences between our reaction and the tuberculin reaction.

With egg-globulin, we obtained similar sensitization to that of the crude egg white. In a small series with crystallin egg-albumin and ovomucoid, no sensitization leading to necrotic reaction was obtained.

Although the observations at our disposal do not permit the identification of the described sensitiveness with the tuberculin sensitiveness, the conclusion seems at hand that the mechanism underlying both is similar. The tuberculous infection creates in an unknown manner the conditions necessary for the development of the local sensitiveness, as well as the conditions for an increased antibody production, which are not specific to a certain antigen. The protein substances of the tubercle bacilli, as any foreign protein substance which is present in the organism, determines the specificity of the sensitiveness. It is a very interesting problem whether in the resistance against the tuberculous infection, which we observe always as the result of a slight infection, the same factors are at work—a non-specific altered reaction of the organism and a specific reaction made possible by the former.

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3218

The Effect on Blood Sugar of Injections of Bacteria.

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Intercurrent infection unfavorably influences the course of diabetes. Graham[1] finds that infection may be instrumental in precipitating the diabetic into a state of coma. Graham[1] and also Rabinowitch[2] report that infection diminishes the potency of insulin. The dosage required to lower the blood sugar to a certain level is much greater in a diabetic in whom infection is present than in one without this complication. Clinical evidence is accumulating with reference to the fact that infection, focal or generalized, may be an etiological factor in *diabetes mellitus.* Rosenow has recently demonstrated a relation between focal infection and this disease.[3]

Infection in the non-diabetic has been reported by several investigators to give rise to hyperglycemia.[4] The invading organism is believed to lower the carbohydrate tolerance.[5] Menten and Manning,[6] and Jeckwer and Goodell[7] have demonstrated hyperglycemia as a result of the injection of some types of bacteria. Thomas[8] observed that guinea pigs in whose organs bacilli of the enteridis-paratyphoid B group were isolated, showed characteristic lesions in the pancreas, such as hydropic degeneration of the islands of Langerhans.

In view of the above facts, a study of the relation to carbohydrate metabolism of bacteria invading the animal organism is of paramount importance. The experiments here reported deal with the effect on the sugar level in the blood of rabbits receiving intraperitoneal injections of three billion heat-killed bacteria. The organisms were obtained from 24-hour cultures. They were removed from their growth media and suspended in saline, and the saline suspensions rendered sterile by heating for several one-hour periods at 60° C. (We are much indebted to the Research Department of Parke, Davis & Co., Detroit, for the growth of the cultures and the preparation of the sterile saline suspensions used in our experiments.) The animals were starved for 18 to 24 hours previous to the administration of the bacterial suspension. Blood sugar was determined by the method devised by Shaffer and Hartmann,[9] and calculations made according to their directions. Temperature readings were taken immediately after the withdrawal of blood from the ear vein.

The results of the work thus far indicate that bacteria may be divided into three groups with relation to their effect on the sugar content of the blood.

Group I. Those That Cause an Increase in Blood Sugar.

To this group belong *Bacillus coli communior, Bacillus coli communis, Bacillus dysentery, Staphylococcus pyogenes aureus, Bacillus paratyphoid A,* and *pneumococcus* type I.

BACILLUS DYSENTERY.

Weight of rabbit, 1750 gm. Amount injected, 3 billion bacteria in 1 cc.

	Blood Sugar	Rectal Temp.
	Per cent	
Control	0.107	100.6
2 hours after injection of bacterial suspension	0.160	104.2
3 hours after injection of bacterial suspension	0.174	105.0

Group II. Those that do not Cause a Change in the Sugar Concentration of the Blood.

In this group are to be found *Bacillus pyocyaneus,, Bacillus pertussis, Friedlander's bacillus, Bacillus diphtheriae, Bacillus influenzae, Bacillus fecales alkaligenes, Streptococcus viridans,* and *Bacillus typhosus.*

BACILLUS ENTERIDIS.

Weight of rabbit, 1200 gm. Amount injected, 3 billion bacteria in 1 cc.

	Blood Sugar	Rectal Temp.
	Per cent	
Control	0.120	101.4
1¾ hrs. after injection of bacterial suspension	0.120	102.0
3 hours after injection of bacterial suspension	0.126	102.0

Group III. Those that Cause a Decrease in Blood Sugar.

Streptococcus hemolyticus is the only one we have thus far found to belong to this group.

STREPTOCOCCUS HEMOLYTICUS.

Weight of rabbit, 1300 gm. Amount injected, 3 billion bacteria in 1 cc.

	Blood Sugar	Rectal Temp.
	Per cent	
Control	0.098	101.5
2 hours after injection of bacterial suspension	0.079	102.0
3 hours after injection of bacterial suspension	0.082	102.0

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3219

Action of Sodium Thiosulfate in Treatment of Metallic Intoxications and Lesions of the Skin.

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Since sodium thiosulfate therapy was first introduced clinically by Dennie and McBride,[1] much interest has centered about its use in metallic toxemias. Given intravenously it has proven efficacious in relieving dermatitis due to heavy metals, especially arsenic and mercury. Myers and Groehl[2] have studied the excretion of arsenic when sodium thiosulfate was given in cases of post-arsphenamine dermatitis. Its use in diagnostic procedure[3] has, in many instances, shown arsenic to be the etiological factor in skin lesions of obscure origin.

The object of this investigation was to study the effect of salvarsan and sodium thiosulfate on the blood chemistry of experimental animals. Chloride and sugar determinations were made, these two substances being chosen as representatives of two main groups of blood constituents, namely electrolytes and nonelectrolytes, and also because they represent the abnormalities in the production of skin lesions as found in eczema in arsenical and mercurial poisoning. In a succeeding publication the action of sodium chloride on the sugar and chlorides will be given, as well as the effect on other blood constituents such are urea, uric acid and the calcium-phosphorus balance.

Whitehorn's[4] method was used for the chlorides, and Hastings and Hopping's[5] modification of McLean's[6] method for sugars. Examination of the blood was always made previous to the injection. The animals were subjected to handling and were made accustomed to their surroundings so that conditions of shock can hardly be considered as playing any part in the taking of the specimens. The blood was obtained from the ear vein.

Salvarsan, in the form of a 0.5 per cent solution of the disodium salt in freshly distilled water, was given intravenously by gravity to rabbits, in doses of 50 mg. per kg. body weight. Table I gives the average values for 7 rabbits for the sugars and chlorides at different intervals after the salvarsan injection.

TABLE I.

Changes in the blood sugar and chlorides after the administration of Salvarsan.

0.5 per cent aqueous solution — Dose: 50 mg. per kg. body weight.
Intravenous injection.

	Initial blood	Blood after intervals of minutes					
		0+	10	20	40	90	180
Sugar—mg./100 cc.	117.4	131.8	126.1	136.0	143.3	125.1	131.6
NaCl—mg./100 cc.	536.9	529.1	539.4	547.7	563.1	592.5	586.8

Sodium thiosulfate was administered intravenously in the form of a 25 per cent freshly prepared aqueous solution. The dose was 700 mg. per kg. body weight. The average blood changes for a group of 9 rabbits are shown in Table II.

TABLE II.

Changes in the Blood Sugar and Chlorides after the Administration of Sodium Thiosulfate.

25 per cent solution — Dose: 700 mg. per kg. body weight.
Intravenous injection.

	Initial blood	Blood after interval of minutes						
		0+	15	30	60	90	180	300
Sugar—mg./100 cc.	129.0	169.0	151.7	152.68	144.46	120.2	130.4	123.6
NaCl—mg./100 cc.	529.9	510.2	539.4	540.74	529.50	539.9	567.7	554.0

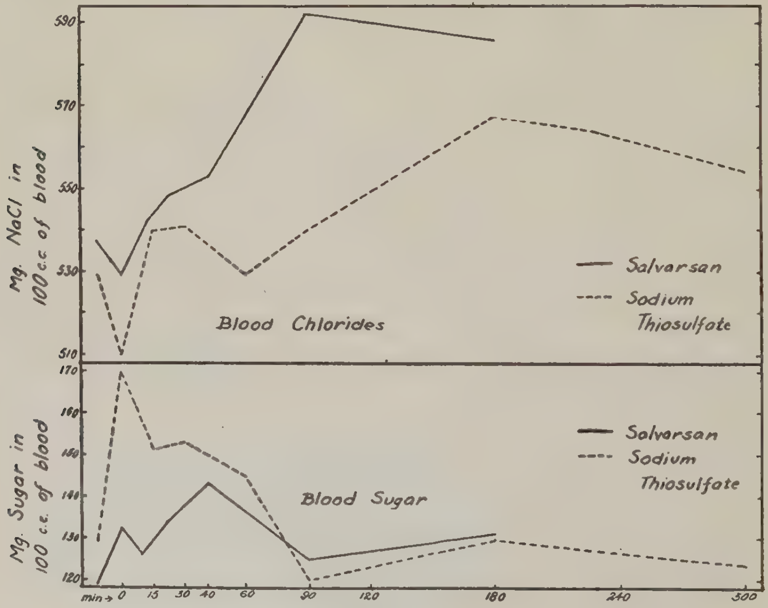

CHART 1.

Chart 1 shows graphically the results of the two types of treatment.

It will be seen that salvarsan and sodium thiosulfate have a parallel effect which exists quite uniformly over a period of 3 to 5 hours. The blood sugar is increased immediately after the injection, and later decreases to the original level or lower. The chlorides, on the other hand, drop first, followed by an increase and then a very slow decrease. A comparison between the blood chlorides and the leucocyte count as carried out by Mueller and Myers[7] shows that for both there is a period of pronounced drop over the same interval. The leucocytes return to normal gradually, but the increase in chlorides extends over a considerably longer period. These results have been checked up clinically, confirming the experimental findings on rabbits.

It may be reasonably assumed that the reason for the effectiveness of sodium thiosulfate in cases of arsenic intoxication is due to a stimulation of the processes which normally take care of arsenic but which have become sluggish on account of the toxic action of the metal. Thiosulfate passes through the same channels as salvarsan, but where the latter intoxicates the cells and retards their action, the former is tonic, rather than toxic, and stimulates the processes by which it, and also the arsenic, may be eliminated.

Mention must be made here of the work done by Mueller and Myers[8] on the mechanism of arsenic lesions. They have demonstrated that skin and liver are both controlled by the autonomic nervous system. All salvarsan injections, with the exception of silver salvarsan, are accompanied by a shock of the involuntary nervous system as evidenced by a decrease in the leucocyte count. In some cases, more susceptible than others to reactions after salvarsan, this leads to an intoxication of the involuntary nervous system. There are two types of skin and liver function to be considered here: first, the prevention of too great quantities of arsenic from entering the cells from the blood stream, and second, the excretion of arsenic that has been deposited in the cells. Intoxication of the autonomic nervous system and consequent slacking of the nerve control of the cells of the skin and liver leads to the following vicious circle: 1. Arsenic from the blood enters the cells in unduly large quantities due to the interruption of the "barrier" function. *2. Cellular activity is so impaired that* *the organs are unable to excrete arsenic in sufficient quantities.* 3. Excessive loading of arsenic in the skin and liver causes injury and even destruction of the cells, producing dermatitis and jaundice. The work of Throne, Van Dyck, Marples and Myers[9] has shown that arsenic may produce lesions of the skin which are only manifest in many instances several years after the initial intoxication. The same authors have demonstrated that arsenic may be an etiological factor in the genesis of eczema.

Sodium thiosulfate, when given intravenously, causes increased arsenic excretion as shown by the urine analyses before and after thiosulfate reported by Groehl and Myers.[2] When this load is removed from the cells and the factor so irritating to the involuntary nervous system is lessened it is probable that this latter is able to resume its normal function of maintaining a barrier and excreting arsenic normally.

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3220

The Hydrogen Ion Concentration of the Nucleus and Cytoplasm of the Egg Cell.

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By means of the microinjection apparatus the series of acid dye indicators of Clark and Lub and the basic dye Neutral Red were injected both into the cytoplasm and into the nucleus of the immature starfish egg.

The color changes upon injection indicate the pH of the nucleus to be in the neighborhood of 7.6 to 7.8, whereas that of the living cytoplasm is 6.6 to 6.8,[1] and cytoplasm cytolyzed by mechanical injury is 5.4 to 5.6.

TABLE I.

Starfish Egg.	Cytoplasm.		Nucleus
	Injured 5.4-5.6	Normal 6.6-6.8	7.6-7.8
Methyl Red	Yellow	Yellow	Yellow
Br. Cr. Purple	Yellow	Blue	Blue
Br. Thy. Blue	Yellow	Green	Blue
Phenol Red	Yellow	Yellow	Red
Cresol Red	Yellow	Yellow	Yellow
Neutral Red		Red	Orange

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3221

The Effect of Fluorides on the Echinoderm Egg.

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In the course of some experiments by one of us on the action of fluorides on coagulation of the blood it occurred to us to note the effect of fluorides on other cell structures. The arbacia egg, since it is reliable and convenient to work with, was chosen for study.

The eggs were placed in 70 cc. of sea water containing one cc. of an isotonic solution of NaF, KF, K oxalate, and K citrate respectively. An immediate flocculation of the eggs occurred in the sea water to which NaF, or KF were added. No similar phenomenon occurred with K oxalate or K citrate, or when artificial sea water was used in which Ca was absent. The flocculation, therefore, seems to be a direct effect of the fluorine ion and is not due to the removal of the calcium from the sea water. The flocculation was characterized macroscopically by the instantaneous clumping of the cells into large masses. Microscopically the groups of cells seemed to be enmeshed in a bed of gelatinous material. No such gelatinous material was noted except in the cases where NaF or KF were added. This phenomenon occurred both in the unfertilized and the fertilized eggs.

No change in the size of the cells was noted for a period of over two hours. The H-ion concentration of the solutions was followed potentiometrically and showed practically no deviation from the normal in all solutions.

Further observations were made on the time and rate of division in the fertilized cell. Isotonic solutions of KF, NaF, K oxalate, and K citrate were added within five minutes after fertilization and the time of the first division and the number of cells going through the first division each minute after this, was noted. It was found that the cells to which KF or NaF had been added divided at about the same rate as the controls. With K oxalate or K citrate the rate of division was very much slower. A slightly greater number of eggs divided with KF or NaF than with the control, and the per cent of division was much reduced with K oxalate or with K citrate.

TABLE I.
Results of a typical experiment.

	Per cent of eggs going through 1st division	Time from fertilization to 1st cell division.	Total time for 1st cell division
Control (sea water)	86	42 min.	8 min.
Sea water + NaF (M/100)	92	42 min.	8 min.
Sea water + KF "	89	42 min.	9 min.
Sea water + K oxalate	61	46 min.	12 min.
Sea water + K citrate	54	45 min.	14 min.

A comparison of the rate of oxidation, determined by the Winkler method on the fertilized cells to which KF, NaF, K oxalate or K citrate were added, showed no constant variation of the rate of oxygen consumption from the normal controls.

3222

The Effect of Nephrectomy and Suprarenalectomy in the Rat on Resistance.

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There is abundant evidence that the suprarenal gland, in some manner still unknown, plays an important rôle in the defensive mechanism of the body against poisons. Lewis[1, 2, 3] in 1921, established the fact that suprarenalectomized rats are killed by much smaller doses of a variety of poisons than are normal animals. These experiments have been confirmed by Scott,[4, 5] Marine and his co-workers,[6, 7] Belding and Wyman,[8] and more recently Jaffe.[9] Stewart and Rogoff,[10] and Rogoff and his co-workers,[11, 12] while clearly showing in their experiments that suprarenalectomized animals have lowered resistance, believe that this is not specifically due to loss of suprarenal substance. They[10, 11] argue as follows: Since the general mortality following double suprarenalectomy in rats obtained by various workers, is about 50 per cent, and since approximately 90 per cent of this mortality occurs between the 5th and 15th day following the operation, therefore any increased sensitiveness found between the 5th and 15th day is due to the moribund state of the animal. This conclusion ignores the well established fact that while 50 per cent of rats survive indefinitely the removal of both suprarenals, practically 100 per cent are killed by a dose of poison (typhoid vaccine), far below the minimum lethal dose for normal animals, and that no other operative procedure has so far been found in which a comparable lowering of resistance follows.

It is our purpose to report further experiments in support of the view that doubly suprarenalectomized rats while still surviving in good condition, have markedly lowered resistance, and also to report experiments showing that rats made truly moribund by nephrectomy do not show a comparable lowered resistance. We, therefore, have studied the resistance of nephrectomized rats to small doses of standard typhoid vaccine. The principal data of these experiments are given in Table I.

TABLE I.
Resistance of Doubly Nephrectomized Rats to Typhoid Vaccine.

Number of rats	Average weight	Interval between removal 1st and 2nd kidney	Amount vaccaine	Duration of Life		
				Min.	Max.	Average
	gm.	days	cc.	hrs.	hrs.	hrs.
5	221	7	—*	72	104	89
6	261 1/2	30	—*	72	102	91 2/3
6	197	7	.4†	80	97	90

*Controls.
†Injected 48 hours after removal of second kidney.

The right and left kidneys were removed at intervals of 7 and 30 days. Forty-eight hours after removal of the second kidney all the rats were semi-comatose and in a moribund state. In spite of this fact the duration of life of the animals injected with 4 cc. typhoid vaccine did not differ in any way from the non-injected rats.

A parallel series of rats of the same stock and age were suprarenalectomized and their resistance tested with small doses of the same standard typhoid vaccine. The principal data of these experiments are given in Table II.

The data of Table II show that doubly suprarenalectomized rats, injected with 0.4 and 1 cc. of typhoid vaccine at 48 hours, 72 hours, 82 hours, and 96 hours after suprarenalectomy are not killed. On the other hand beginning about 144 hours after suprarenalectomy all rats injected were killed by 0.4 to 1 cc. of the poison, whereas the mortality from control suprarenalectomized rats in our series has been approximately 30 per cent. These results clearly indicate that the lowering of resistance following suprarenalectomy is delayed at least 4 days, and that the rather abrupt fall in resistance after this time is due to the exhaustion of the influence normally exerted by the suprarenals. This sharp change in resistance after 96 hours can be interpreted as indicating that the suprarenal influence persists and is effective in the tissues for at least 96 hours. In this respect the suprarenal gland appears to be comparable to the thyroid and parathyroid glands. Thus it is well known that following thyroidectomy no significant change in heat production ordinarily occurs for 4 or 5 days, at which time the decrease is abrupt.[13] A similar factor of safety has been noted in the case of the parathyroid gland. In animals deprived of a normal calcium intake, fatal tetany may occur within a few hours, whereas in dogs fed with bone meal prior to parathyroidectomy, the onset of tetany may be delayed for more than 48 hours.[14]

TABLE II.
Resistance of Doubly Suprarenalectomized Rats to Typhoid Vaccine.

Number of rats	Average weight	Interval* between operation and injection	Amount vaccine	Duration of life after injection.
	gm.	hrs.	cc.	
3	238	48	.4	No immediate effect. Lived 64, 66, 51 *days* respectively.
4	115	72	.4	No immediate effect. Alive after 5, 5, 5, 5 *days* respectively.
4	165	82	.4	No immediate effect. Lived 27, 28, 8, 23 *days* respectively.
2	125	96	1	No immediate effect. Alive after 5, 5 *days* respectively.
4	180	96	.4	No immediate effect. Alive after 5, 5, 5, 5 *days* respectively.
4	172	144	.4	Severe reaction. Dead after 9, 5½, 7, 8 *hours* respectively.
3	122	147	1	Severe reaction. Dead after 6, 9, 12 *hours* respectively.
3	93	168	1	Severe reaction. Dead after 8, 12, 22 *hours* respectively.
4	148	168	.4	Severe reaction. Dead after 6, 13½, 9, 12 *hours* respectively.
2	111	192	.4	Severe reaction. Dead after 10, 20 *hours* respectively.

*Both suprarenals removed at once.
Normal animals withstand 20 cc. of single intraperitoneal injections of standard typhoid vaccine.

While suprarenalectomized animals show a marked lowering of resistance to various poisons, it is known that such animals (rabbits, rats) are capable of producing more antibodies (hemolysins, agglutinins) from the same doses of antigen than are normal animals,[6, 7] and also that such animals show a notable regeneration at the thymus and lymphoid tissues. These striking biological facts, we believe, furnish additional evidence, opposed to the view that the lowered resistance is due to a moribund state of the animal.

SUMMARY.

It has been shown that the duration of life of nephrectomized rats is not affected by injections of the same typhoid vaccine which regularly shortens the life of suprarenalectomized rats. It has also been shown that suprarenalectomized rats have a refractory period of approximately four days before any marked drop in resistance is manifest. These findings, together with the fact that the suprarenalectomized animals fully retain their ability to form antibodies and to regenerate the thymus and lymph tissues, suggest that suprarenalectomy exerts a specific physiologic influence on resistance.

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[1] Lewis, S. T., *Compt. rend.*, 1921, lxxiv, 163.

[2] Lewis, S. T., *Compt. rend.*, 1921, lxxv, 685.

[3] Lewis, S. T., *Am. J. Physiol.*, 1923, lxiv, 506.

[4] Scott, W. S. M., *J. Exp. Med.*, 1923, xxxviii, 543.

[5] Scott, W. S. M., *J. Exp. Med.*, 1924, xxxix, 457.

[6] Take, N. M., and Marine, D., *J. Infec. Dis.*, 1923, xxxiii, 217.

[7] Jaffe, H. L., and Marine, D., *J. Infec. Dis.*, 1924, xxxv, 334.

[8] Belding, D., and Wyman, L. C., *Am. J. Physiol.*, 1926, lxxviii, 50.

[9] Jaffe, H. L., *Am. J. Path.*, 1926, ii, 421.

[10] Stewart, G. N., and Rogoff, J. M., *J. Pharmacol. and Exp. Therap.*, 1922, xix, 97.

[11] Rogoff, J. M., and DeNecker, S., *J. Pharmacol. and Exp. Therap.*, 1926, xxvi, 243.

[12] Rogoff, J. M., and Echer, E. E., *Arch. Path. Lab. Med.*, Soc. Trans., 1926, i, 309.

[13] Marine, D., *Arch. Int. Med.*, 1923, xxxii, 811.

[14] Marine, D., *J. Exp. Med.*, 1914, xix, 89.

3223

Fractionation of Irradiated Cholesterol: Chemical Observations.

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One of us recently reported[1] that cholesterol, as a result of exposure in air to light from the mercury-vapor quartz lamp, reacted differently from the untreated cholesterol towards a reagent consisting of aniline and concentrated hydrochloric acid. With the aid of this color reaction, fractionation of irradiated cholesterol was undertaken. It was hoped that in this way an antirachitically active fraction might be obtained.

After repeatedly recrystallizing irradiated cholesterol, it gave a negative color reaction, while the mother liquor, from the first recrystallization, gave a more intense reaction than did the irradiated cholesterol itself. The mother liquor was concentrated by evaporation and filtered. The final filtrate was yellow, removal of the remaining solvents on a water bath left a yellow oil. Recrystallization of purified cholesterol gave no such product. For convenience, it will be referred to as "U. V. oil of cholesterol" until a more suitable name is proposed.

The yield of crude oil was about 10 per cent when the cholesterol was irradiated for two hours, and about 5 per cent when irradiated for one hour. The irradiation was performed in air at a distance of 18 inches, using a Hanovia lamp. The cholesterol was removed by the digitonin precipitation method. About 40 per cent of the crude oil was recovered as cholesterol-free oil.

The color reactions usually used for the detection of cholesterol were tried on the cholesterol-free oil. With 1 mg. of this oil, a positive Salkowski test was obtained; with 1 mg. of purified cholesterol the upper layer was yellow, and very little green was obtained in the lower layer. Apparently the Salkowski[2] test is not given by purified cholesterol in small quantities while it is given intensely by one or more of its derivatives. The U. V. oil, both crude and cholesterol-free, gave a positive Liebermann-Burchard reaction, although the green was of a somewhat different tint from that given by cholesterol. Cholesterol gave a negative Lifshütz test for oxycholesterol, while a positive test was obtained with irradiated cholesterol and with both the crude and cholesterol-free U. V. oils. With the aniline-hydrochloric acid reagent, both the crude and the cholesterol-free U. V. oils gave positive tests for the chromogenic substance.

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[1] Shear, M. J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 546.

[2] Salkowski, E., *Arch. f. d. g. Physiol.*, 1872, vi, 207.

3224

Fractionation of Irradiated Cholesterol: Antirachitic Potency of the Fractions.

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There have been a number of attempts to separate an antirachitically potent fraction from cholesterol irradiated in air. Hess, Weinstock and Sherman,[1] and Rosenheim and Webster[2] tried without success. The latter authors claim to have isolated an active fraction from cholesterol irradiated in nitrogen, but their experimental data do not seem to warrant such a claim. Nitzescu and Popoviciu[3] reported the separation of irradiated cholesterol (presumably irradiated in air) into an active and an inactive fraction.

The preceding paper[4] is a report of the separation of a yellow oil formed from cholesterol as a result of irradiation in air. The antirachitic potency of this "U. V. oil of cholesterol" was tested by means of curative experiments on rachitic rats, in which the material tested was incorporated in the diet. At the May, 1926, meeting of the American Pediatric Society, one of us, in discussing a paper by Schlutz and Ziegler, reported[5] that this material cures rickets in rats. During this same discussion, Julius H. Hess[6] stated that Koch had obtained an active extract from irradiated cholesterol.

The present communication is a brief report of the results so far obtained. The new points brought out by this investigation, in which more than 100 rats were used, are as follows:

1. Crude "U. V. oil of cholesterol" cures experimentally produced rickets in rats.
2. Cholesterol-free "U. V. oil of cholesterol" cures experimentally produced rickets in rats.
3. The antirachitic effect of the U. V. oil is not due to material extracted by ether from the reagent employed for the removal of the cholesterol.
4. Cholesterol-free "U. V. oil of cholesterol," protected by cottonseed oil, and incorporated in the diet, retains its antirachitic potency after two months' storage.

The table gives a summarized statement of some of the experiments.

TABLE I.

Date of autopsy	No. of rats	Test material added to diet	Histological Findings
5/27/26	4	2% crude U. V. oil and 3% cottonseed oil	Advanced healing
,,	2	3% cottonseed oil (controls)	No healing
7/2/26	6	0.1% cholesterol-free U. V. oil	Moderate healing
,,	6	0.2% recrystallized irradiated cholesterol	No healing
,,	5	controls	No healing
7/19/26	5	0.3% crude U. V. oil and 3% cottonseed oil	Advanced healing
,,	5	0.3% cholesterol-free U. V. oil and 3% cottonseed oil	Advanced healing
7/23/26	6	controls	No healing
8/2/26	5	3% cottonseed oil (controls)	No healing
9/26/26	4	ether extract of digitalin	No healing
,,	2	0.3% cholesterol-free U. V. oil and 3% cottonseed oil (stored 2 months)	Advanced healing
,,	2	controls	No healing

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[1] Hess, A. F., Weinstock, M., and Sherman, E., *J. Biol. Chem.*, 1925, lxvi, 145; *ibid*, 1926, lxvii, 413.

[2] Rosenheim, O., and Webster, T. A., *Biochem. J.*, 1926, xx, 537.

[3] Nitzescu, I. I., and Popoviciu, G., *Compt. rend. Soc. biol.*, 1926, xciv, 1301.

[4] Shear, M. J., and Kramer, Benjamin, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 51.

[5] Kramer, Benjamin, *Am. J. Dis. Child.*, 1926, xxxii, 466. (Society Transactions).

[6] Hess, J. H., *ibid.*

3225

Antirachitic Properties of Cod Liver Oil Concentrate.

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Cod liver oil causes retention of lime salts and also cures and prevents rickets when administered orally. The assumption, therefore, was made that the beneficial effects of this agent result from its acting directly on the mucosa of the gastro-intestinal tract. Thus, Zucker and Matzner[1] were unable to obtain healing in rachitic rats with a subcutaneous injection of an extract of cod liver oil, although when administered orally it was invariably potent. Similarly, Lesne and Vogliano[2] failed to cure rickets in rats when cod liver oil was injected hypodermically. On the other hand, Soames[3] reported moderate healing of rickets when cod liver oil was injected either subcutaneously or intraperitoneally. Hess, Weinstock and Hellman[4] were successful in curing rickets with an aqueous suspension of irradiated cholesterol administered subcutaneously; and Wilkins and Kramer[5] have shown that an ether solution of a concentrate of cod liver oil is capable of curing severe rickets in children.

The experiments briefly reported here may shed some light on the discrepancies in the literature just mentioned. The results of these experiments, in which 60 rats were used, are as follows:

1. The unsaponifiable fraction* of cod liver oil from which most of the cholesterol had been removed (crude concentrate) cured experimental rickets within 14 days when injected subcutaneously in ether solution. The same result was obtained when it was incorporated in cottonseed oil and added to a rickets-producing diet. The curative dose was equivalent to 2 cc. of cod liver oil.

2. When entirely freed from cholesterol by means of digitonin precipitation, an active concentrate was obtained which cured experimental rickets both when fed, and when injected in ether solution. The curative dose was equivalent to 2 cc. of cod liver oil.

3. When palmitin was used instead of ether as the vehicle for injection, healing did not occur when the same dose was administered.

The table gives a summary of some of the experiments.

These observations indicate that the antirachitic substance is as potent when injected subcutaneously as when given orally, provided a suitable vehicle is used.

This potent cholesterol-free concentrate was tested with the aniline hydrochloric acid reagent. It gave a decidedly positive reaction.

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* Supplied through the courtesy of Dr. Harry E. Dubin of H. A. Metz Research Laboratory.

TABLE I.

Date of autopsy	No. of rats	Test material	Histological Findings
7/23/26	5	Non-sapon. fraction of cod liver oil, +3% olive oil; in diet	Advanced healing
,,	5	Crude extract; injected in ether solution	Advanced healing
,,	5	Controls	No healing
9/12/26	5	Cholesterol-free extract; injected in ether solution	Advanced healing
,,	2	Controls; injected with ether	No healing
,,	6	Cholesterol-free extract; injected in palmitin	No healing
,,	3	Controls; injected with palmitin	No healing
,,	6	Controls; no injection	No healing
,,	6	Cholesterol-free extract, +3% cottonseed oil; in diet	Moderate healing
,,	6	Controls; 3% cottonseed oil in diet	No healing

[1] Zucker, T. F., and Matzner, M. J., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 186.

[2] Lesne, E., and Vaglianos, M., *Compt. rend. Acad. d. Sc.*, 1924, clxxvii, 711.

[3] Soames, K. M., *Biochem. J.*, 1924, xviii, 1349.

[4] Hess, A. F., Weinstock, M., and Hellman, F. D., *J. Biol. Chem.*, 1925, lxiii, 305.

[5] Wilkins, L., and Kramer, B., (unpublished work).

3226

Antagonism of Barium Chloride and Glandular Extracts on Chromatophores by Epinephrin and Ephedrine.

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In the course of a comparative study of the effect of ephedrine and epinephrin on the chromatophores of the Squid, *Loligo Pealii,* it was observed that these drugs antagonize the stimulating action of barium chloride, the extracts of the parathyroid and anterior as well as the posterior lobe, pituitary gland. Epinephrin is not effective as a rule after barium chloride in mammals. Roth[1] described a good case of such action in the frog. Fawcett, Beebe, et al.,[2] found that the stimulation by the extracts of various glands on the uterus of the cat could likewise be inhibited by epinephrin. It is therefore interesting to note that invertebrate smooth muscle cells which appear to be under the control of the sympathetic nervous system exhibit actions similar to the above.

It was found that epinephrin is about twenty times as effective as ephedrine in antagonising the action of the extracts of the parathyroid and the anterior lobe of the pituitary gland; twice as effective in antagonising the action of the extract of the posterior lobe of the pituitary gland. Ephedrine is almost ten times more effective than epinephrin in antagonising barium chloride. (This work will be published in full elsewhere.)

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[1] Roth, G. B., *Arch. Internat. Pharm. et Therap.*, 1923, xxvii, 333; PROC. SOC. EXP. BIOL. AND MED., 1922-23, xx, 43.

[2] Fawcett, G. G., Beebe, S. P., et al., *Am. J. Physiol.*, 1915, xxxvii, 453; xxxix, 154.

3227

Influence of Salts and Acids on Penetration of Brilliant Cresyl Blue into the Vacuole.

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The theory[1] underlying the experiments described in the present paper is that the basic dye, brilliant cresyl blue, exists in two forms which we may call DB, a free base (predominant at higher pH values), and DS, a salt (predominant at lower pH values). It is assumed that the living cell of *Nitella** is permeable primarily to DB, and only very slightly to DS. Thus when we speak of the entrance of the dye into *Nitella* we refer to penetration of DB and not of DS. At equilibrium DB in the vacuole is equal to or proportional to DB in the external solution, and the amount of the total dye (DB and DS) in the sap depends on the extent to which DB changes to DS in the sap.

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* The *Nitella flexilis* used is a fresh water plant, with multinuclear cells about four or more inches in length, having an outer cellulose wall, a very thin protoplasmic layer, and a relatively large vacuole filled with sap at about pH 5.5, containing about 0.1 M halides, protein, potassium, and other substances.

The rate of penetration of DB into the vacuole may depend on conditions in (1) the external solution, (2) the protoplasm, (3) the vacuole. It is assumed[1] that under varying conditions the seat of the controlling factor may change. One of the important problems in permeability is to locate this controlling factor. An attempt to throw light on this question is made in the present paper by exposing living cells to certain salts and acids and determining the rate of penetration of dye into the vacuole, when the cells are subsequently placed in a solution of dye.

1. *Effect of Chlorides with Monovalent Cations on the Cell.*

Cells were placed in 0.03 M NaCl solution[2] for 10 minutes, after which they were removed, wiped, and washed in distilled water (pH 5.4) for 5 seconds, then again wiped, and placed in 0.00007 M dye solution[3] at pH 7.7 (M/150 borate buffer solution). The rate of penetration of dye after 2 minutes was found to be about 75 per cent less than in the control cells which were transferred directly from distilled water to the same dye solution. This decrease is not due to the direct effect of NaCl on the dye, since, when the cells are placed in the 0.00007 M dye solution at pH 7.7, containing 0.03 M NaCl, the rate of penetration of dye is slightly higher than that of the control cells placed in dye solution containing no NaCl. Nor is this decrease due to the adhering of NaCl to the cell wall in such fashion that it cannot be washed or wiped away, because no decrease is found to take place if we dip the cells in NaCl solution for a few seconds, instead of for 10 minutes. No change in the concentration of halides is found to take place in the sap after the cells have been exposed for 10 minutes to NaCl solution.

These results indicate that the decrease in the rate of penetration of dye results from the effect of NaCl on the protoplasm (either at the surface or interior). The same result is obtained when KCl and LiCl are used.

2. *Effect of Chlorides with Bivalent and Trivalent Cations.*

The cells were exposed as before to solutions of chlorides with bivalent cations, $MgCl_2$ and $CaCl_2$, and with a trivalent cation, $LaCl_3$. In contrast to the chlorides with monovalent cations, there was with these salts no decrease in the rate of penetration of dye when cells were previously exposed to the same series of concentrations of salt solutions for 10 minutes whereas there was an increase (about 10 per cent) in the rate of penetration of dye when the cells were placed directly in the 0.00007 M dye solution at pH 7.7 containing 0.003 M salt.

The effect of these salts on the dye, increasing penetration, is not sufficient to mask entirely the possible effect of the salt on the protoplasm, decreasing penetration, in case the latter is equal to that of the chlorides with monovalent cations, so that there is a difference in the behavior of the chlorides with monovalent cations on the one hand, and with bivalent and trivalent cations on the other, toward the protoplasm.

3. *Effect of Salts with Different Anions.*

By using the same experimental procedure as before we find that $NaNO_3$ and Na_2SO_4 give the same result as the chlorides with monovalent cations, while $MgSO_4$ and $La(NO_3)_3$ behave like $MgCl_2$ and $LaCl_3$.

These experiments show that specific effects of these salts either on the protoplasm or on the dye are due chiefly to the cations.

4. *Effect of Buffer Mixtures and Acids on the Cell.*

What happens if we place cells in a buffer solution which contains sodium or potassium salts at the same pH value as the control? When cells are placed in a phosphate buffer solution at pH 7.7 for 10 minutes and then transfrered to the dye solution the rate of penetration of dye is about 30 per cent lower than the rate of the control (cells directly transferred from tap water at pH 7.7 to the same dye solution). With borate buffer solution at pH 7.7 there is no change from that of the control. Since the pH value is the same in all cases, this decrease in the rate with the cells exposed to the phosphate buffer mixture cannot be due to the effect of H or OH ions on the cell, but is due to some other cause.

It is not due to a specific effect of potassium because the same result is obtained whether we use the phosphate buffer mixture made up with Na_2HPO_4 containing KH_2PO_4 or NaH_2PO_4.

It is not due to the effect of the phosphate buffer mixture on the dye because the rate of penetration is the same whether the cells are placed for 1 minute in the dye solution at pH 7.7 made up with borate or phosphate buffer mixture.

It may be due to phosphoric acid, which enters the protoplasmic surface as undissociated molecules and dissociates after entrance, thus, lowering the pH value of the protoplasm. This lowering of the pH value would reduce the amount of DB in the protoplasm as compared to the control, thus resulting in a decrease in the rate of penetration of DB from the protoplasm into the vacuole.

When cells are exposed for 10 minutes to solutions at pH 4 (1) of hydrochloric acid, (2) of boric acid, (3) of phosphoric acid, and then placed in the dye solution, the rate of penetration of dye as compared with the control is the same with (1) and (2), but it is about 20 per cent lower with (3). It would appear from (1) that H ions do not enter the protoplasm, while (2) indicates that if undissociated molecules of boric acid enter and dissociate it is too weak an acid to affect the pH value of the protoplasm, whereas (3) indicates that phosphoric acid enters and dissociates sufficiently to cause a decrease in the pH value thus decreasing the rate of penetration of dye into the vacuole or acts on the surface.

It may very well be that the effect of the phosphate buffer solution on the protoplasm is twofold, (1) due to phosphoric acid,[4] (2) due to the Na and K salts, in view of the fact that Na and K aschlorides have considerable effect on the cell.

The fact that sodium borate has no effect cannot be due entirely to its low concentration since the concentration of Na is about 0.01 M at pH 9, at which concentration NaCl has a marked inhibiting effect, and yet there is no effect of the sodium borate. Whether this is due to the masking of the decreasing effect of Na by an opposite action of borate ions or due to the effect of high pH value it is at present undecided.

5. *Effect of Acetic Acid on the Cell.*

These results may help to explain the reason why a decrease[5] in the rate of penetration of dye takes place when cells are exposed to an acetate buffer solution in which the pH value of the sap is lowered, though according to the theory[1] already outlined we might expect an increase in the rate of dye penetration to take place. If we suppose that the increase of penetration due to lowering of pH value of the sap is masked by the decreasing effect of (1) sodium acetate on the protoplasm, and

(2) of acetic acid either on the protoplasm or on the "film of liquid" between the protoplasmic surface and the cell wall, then we might expect a decrease in the rate of penetration, on the basis of the theory[1] mentioned above.

This decrease in the rate of penetration of dye is not caused by the lowering of the pH value of the external solution outside the cell wall as a result of diffusion of acetic acid, since stirring the solution does not change the relative decrease in the rate of penetration of dye (on comparing the rate of the test experiment with that of the control experiment when in both cases the solutions are stirred).

When the living cells of *Valonia macrophysa* are exposed for 1 hour to sea water at pH 5.9 containing (1) acetic acid in which the pH value of the sap is lowered, (2) hydrochloric acid, in which the pH value remains unchanged, and subsequently transferred to the sea water containing 0.00007 M dye, after 20 minutes the rate of penetration is found to be much lower than that in the case of the control experiment (cells directly transferred from sea water to the same dye solution). The rate of penetration of dye is found to decrease (1) when cells are previously exposed to sea water at pH 8.1 containing 0.003 M NH_4Cl until the pH value of the sap is increased, and then transferred to the sea water containing dye, (2) when cells are placed in sea water at pH 8.1 containing dye and 0.003 M NH_4Cl.

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[1] Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 75.

[2] Different concentrations of NaCl solution were used (from 0.05 M to 0.0006 M). It was found that there was no decrease at 0.0006 M salt solution. The decrease caused by an exposure of cells to 0.03 M salt solution cannot be due to injury, though the cells can live in this solution only a few hours, since the same type of decrease may be obtained in the salt solution, at 0.005 M for example, in which the cells can live for several days.

[3] Solutions are not stirred unless otherwise stated.

[4] Another way of interpreting this decrease is as follows: The pH value of the very thin film of liquid between the protoplasmic surface and the cell wall is lowered as a result of diffusion of phosphoric acid from the protoplasm into this film when cells are transferred from the phosphate buffer solution to the dye solution, but at pH values of the phosphate buffer solution above pH 7 the decrease in the rate of penetration of dye cannot be attributed to this, since the pH value of the solution to which the cells are previously exposed is higher than the pH value of the dye solution.

[5] The writer has found that in some cases there is an increase in the rate of penetration of dye instead of a decrease. Such a difference may be due to the difference in the condition of the protoplasm. Irwin, M., *J. Gen. Physiol.*, 1925-26, ix, 561.

3228

Endocrine Glands of Rabbits Fed Cod Liver Oil or Irradiated Cholesterol.

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Studies on the indirect effects of ultra violet light on growth, and its therapeutic action in rickets and tetany in children have received new impetus recently from the independent observations of Hess and Weinstock[1], and of Steenbock and his collaborators[2] that diets which are deficient in certain respects may be made complete by irradiation with light of short wave lengths, or by the addition of certain irradiated substances, such as cholesterol.

The light of the quartz mercury vapor lamp has been found to have definite secondary effects upon some of the endocrine tissues, also, producing a notable hypertrophy of the hypophysis and the (external) parathyroid glands of normal rabbits[3]. The question arises whether the affected glands mediate some of the metabolic effects ascribed to the radiations, and whether irradiated food materials, which appear to have effects similar to those of ultra violet light on growth and in disturbances of calcium and phosphorus metabolism, also act by a specific stimulation of certain endocrine glands.

In these experiments we fed Norwegian cod liver oil, or irradiated cholesterol, to adult male rabbits for periods of several weeks, and at autopsy compared the weights of certain of their endocrine organs with those of normal rabbits, and of rabbits irradiated with a quartz mercury vapor lamp over similar periods of time.

Experiments I and II. Cod liver oil.

In addition to their regular diet, two groups of 6 and of 10 normal adult male rabbits (one group studied in April, the other in December) received 2 to 4 cc. of cod liver oil daily for periods of 3 to 4 weeks. Two groups of 17 and 14 normal rabbits, from the stock of Drs. Brown, Pearce, and Van Allen, examined coincidently with the experimental animals, served as controls. Serum calcium and inorganic phosphorus determinations were made before and at intervals during the experiments, and at autopsy, after exsanguination under ether anesthesia, the weights of the endocrine tissues were determined in proportion to net body weight.

TABLE 1.
Cod liver oil administration.
Average weights of endocrine glands (mgm. per kg. net body weight).

Exp. No.	Group	No.	External parathyroids	Pineal	Hypophysis	Thyroid	Thymus	Adrenals	Testicles
1	Fed cod liver oil	6	5.98	9.0	14.0	103.8	1514.	161.5	2314.
April	Controls	17	6.03	9.5	15.8	158.9	1502.	177.5	2490.
	Irradiated	9	7.21	8.7	19.6	229.3	1082.	200.7	2639.
2	Fed cod liver oil	10	5.87	9.7	16.6	97.1	1252.	245.6	3042.
Dec.	Controls	14	5.94	8.3	14.6	108.0	1341.	207.4	2690.
Oct.	Irradiated	6	8.7	10.8	15.5	102.0	1137.	224.7	2643.

The essentially similar results of these two experiments are shown in Table 1, in which the gland weights of two groups of irradiated rabbits are included for comparison. The feeding of cod liver oil did not change the proportionate weights of any of the endocrine organs beyond the limits of normal individual variation. The weight of the thyroid gland especially varies widely in different rabbits and at different seasons, and the differences between the two experiments in the averages for thymus, adrenals, and testicles are also an illustration of seasonal variation so extensively studied by Drs. Brown, Pearce, and Van Allen[4].

Slight variations in the serum calcium and inorganic phosphorus levels of the experimental rabbits all came within normal limits also.

Experiment III. Cholesterol.

Following the method of Hess and Weinstock, a 1 per cent suspension of Kahlbaum's cholesterin in distilled water was prepared fresh each day and exposed to the unfiltered radiations of a quartz mercury vapor lamp (67 v. 5.5 amp.) for 30 minutes at a distance of 30 cm. The irradiated cholesterin suspension was then fed by stomach tube, 6 days a week, in doses of 10 cc.

(0.1 gm.) to 6 normal, adult, male rabbits. Control groups of 6 rabbits each were fed similar doses of non-irradiated cholesterin, or given only the regular diet of hay, oats, and cabbage. Serum calcium and inorganic phosphorus determinations were made at intervals, and after 4 weeks the animals were anesthetized, exsanguinated, and the weights of certain endocrine glands recorded at autopsy.

TABLE II.
Cholesterol administration.
Average weights of endocrine glands (mgm. per kg. net body weight).

Group	No.	External parathyroids	Pineal	Hypophysis	Thyroid	Thymus	Adrenals	Testicles	Spleen
Fed irradiated cholesterol (May)	5*	7.97	10.4	15.5	99.9	769.6	233.6	2290.	643.4
Fed non-irradiated cholesterol	5*	6.4	8.8	15.54	141.9	1190.	255.8	2258.	538.9
Controls	6	8.66	8.63	15.53	107.	1222.	256.6	2312.	516.6
Irradiated rabbits (June)	6	9.67	9.2	17.95	103.9	971.3	—	2258.	—

*Excluding two rabbits which died during the experiment.

On the basis of this single experiment, attention is directed only to the figures for external parathyroids and hypophysis, in which the hypertrophic effects of ultra violet irradiation have been repeatedly observed. The external parathyroid glands of the rabbits fed irradiated cholesterol were considerably larger than those of the non-irradiated cholesterol group, but a similar (seasonal) parathyroid enlargement had occurred in the normal controls also. The question is: why did the rabbits fed non-irradiated cholesterol not show the usual seasonal parathyroid hypertrophy?

As in the experiments with cod liver oil, no significant progressive variations from normal levels were found in the calcium and inorganic phosphorus levels of the blood of these experimental animals.

Under these experimental conditions, the feeding of cod liver oil or irradiated cholesterol to normal rabbits did not produce changes in the relative weights of endocrine organs, notably the external parathyroids and the hypophysis, which are characteristic effects of irradiation with ultra violet light.

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Western New York Branch

New York State Agricultural Experiment Station, Geneva, N. Y., May 22, 1926.

3229

The Distribution of Dihydrositosterol in Plant Fats.

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In previous reports from this laboratory it has been shown that a saturated sterol, dihydrositosterol, $C_{27}H_{47}OH$, is a constituent of the unsaponifiable matter obtained from the bran and endosperm of corn[1] and wheat.[2] The present communication supplies some additional information on the method of isolation, its occurrence in other plant fats, and properties.

For the isolation of this saturated sterol the method of Anderson and Nabenhauer[3] has been utilized with certain modifications which result in an increased yield of dihydrositosterol. The most important change, in the method already described, is acetylation of the sterol mixture before treating its carbon tetrachloride solution with acetic anhydride and sulfuric acid. This treatment with acetic anhydride and sulfuric acid is repeated until the carbon tetrachloride layer is colorless and no green color is developed by the addition of a fresh portion of acetic anhydride and sulfuric acid. This acetylation prevents the formation of sterol sulfates or sulfonic acids which not only reduce the yield but cause the formation of troublesome emulsions during subsequent washing of the carbon tetrachloride solution.

During the past two years the unsaponifiable matter prepared from a number of plant fats has been examined and dihydrositosterol was found to be present in appreciable quantity in the oil, bran and endosperm of corn, the bran, endosperm and germ of wheat and the bran from rice.

In the case of corn and wheat a higher concentration of the reduced sterol was found in the outer covering, but a comparison between cottonseed oil and cottonseed meal, or linseed oil and linseed meal showed no such concentration.[4]

The last plant fat studied was that extracted from wheat germ. The crystalline sterol from this oil is generally mentioned in the literature as the best known, and fully described by numerous investigators as a homogeneous sterol—sitosterol.[5] We have found that this crystalline sterol is actually a complex mixture of several sterols, one of which is dihydrositosterol.

The dihydrositosterols obtained from these different sources appear to be identical, although there are some slight variations in their physical properties. The substance melts at 143-4° C. and has a specific rotation in chloroform of +24.00 to +25.82. The acetate melts at 140-141 and its rotation is +14.00 in chloroform.

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[2] Anderson, R. J., and Nabenhauer, F. P., *ibid.*, 1924, xlvi, 1717.

[3] Anderson, R. J., and Nabenhauer, F. P., *ibid.*, 1924, xlvi, 1957.

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3230

Hemophilia in Cats After Denervation of the Liver.

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This is to report the occurrence of a phenomenon, resembling hemophilia in cats that have survived an operation in which everything in the hepatic pedicle except the bile duct, hepatic artery and portal vein, is cut. This is spoken of here as "denervation," because the operation was undertaken in connection with some other work in which the function of the hepatic nerves was being investigated. It may be, however, that the severance of the lymphatics from the liver, or some other as yet unsuspected result of the operation, is of more importance in this connection than the cutting of the nerves. But, whatever may ultimately prove to be the real cause of the condition, it has been observed in eight cats that the operation is followed by marked change in the coagulability of the blood. Two of the eight cats died after two and three days of continuous bleeding from a needle prick in an ear vein; a third cat had the bleeding from a similar wound stopped only by the local application of cephalin after the cat had been bleeding for two days. This phenomenon occurred in these cats 4 to 6 weeks after the operation, when they had regained their original weight and were apparently in good condition. In the remaining 5 cats the condition was not as severe, but with them it was often difficult to determine the clotting time of the blood because only a thin clot would be formed. We are of the opinion, however, that the clotting time is practically normal, although the amount of fibrin formed is decidedly less than normal. Unfortunately, there has been no time for further analysis and this preliminary report is made because of the interest of this condition in connection with the problem of hemophilia.

3231

A Study of the Rous Chicken Sarcoma No. 1.

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Gye[1] concluded that malignant growth is a specific disease caused by a living organism. His crucial experiment was carried out as follows. A piece of chicken sarcoma was placed into a culture medium composed of Hartley's broth, 0.2 per cent KCl and rabbit serum, and was incubated at 37° C. After several days the supernatant fluid of this "culture" was no longer able to produce tumors in chickens. Next a piece of fresh tumor was ground up with sand and extracted with salt solution. The clear filtrate was then treated with chloroform. After removal of the chloroform *in vacuo* the filtrate proved to be inactive. However, if a mixture of chloroformed filtrate and culture fluid was injected into chickens, a typical sarcoma was produced. Gye assumed that the living organism which was supposed to have multiplied in the culture was in itself ineffective. It became active only when aided by a specific chemical factor, which was present in the chloroform treated filtrate. According to Gye, the chloroform treatment of the fresh tumor extract destroys the living organism contained in it, but leaves the chemical factor unimpaired. The present experiments deal with the conditions under which reactivation occurs and with the question whether "cultures" of other tissues than maligant tissue contain reactivating substances.

EXPERIMENTAL.

Gye's prescriptions, unless stated otherwise, were exactly followed and need, therefore, not be repeated here. It soon developed that the chief difficulty was the proper chloroformation of the sand filtrate. After three and one half hours incubation of 10 cc. filtrate at 37° C. with 0.2 cc. chloroform, the filtrate was generally inactive in doses of 1 cc. that were used by Gye, but it produced tumors when 2 to 5 cc. were injected. Since Gye's method of chloroformation seemed unsatisfactory, when applied to our tumor material, various changes were tried. It was found that the amount of chloroform used, and the number of times that the filtrate was mixed with the chloroform during the course of the incubation had a decided influence on the inactivation. The technique finally adopted was as follows: 10 cc. lots of clear sand filtrate were placed in test tubes kept in a water bath at 37° C. One cc. of chloroform was added and the filtrate mixed four times during 2 hours of incubation.

The next step was the preparation of the tumor cultures. All media used and all cultures made were tested repeatedly for sterility. It was found, in confirmation of Gye's results, that when 0.5 to 1 gm. of chicken sarcoma were placed in test tubes containing 6 cc. of rabbit serum KCl broth and incubated aerobically at 37° C., the clear supernatant fluid still produced tumors after 3 to 5 days incubation. Here again the dose of the supernatant fluid that was injected was found to be of importance. Cultures that proved to be inactive in the doses used by Gye gave rise to tumors, when larger doses were administered. Therefore, only 6 to 8 days old cultures were used. Similar cultures were also prepared using the same medium and about 1 gm. of 14 days old chicken embryo.

Since so many uncertain factors seemed to play a rôle in the reactivation experiment of Gye, rather extensive control tests were necessary. It should be noted that there exists a difference in the susceptibility of the chickens for tumor material of a low virulence. It seemed, therefore, preferable to test the chloroformed filtrate, the supernatant fluid of the tumor culture, and the mixture of both on the same chicken, instead of using different chickens, as was done by Gye. Furthermore, different untreated sand filtrates, though prepared in exactly the same way, showed a variation in their activity.

In 11 reactivation experiments only 1 positive result was obtained. The course of these experiments was as follows: Untreated sand filtrate was tested on 1 chicken in doses of 0.05, 0.2, 0.5 and 1 cc. In the musculature of both wings and both breasts of 3 to 5 Plymouth Rock hens were then injected 5 cc. of chloroformed sand filtrate, 5 cc. of the supernatant fluid of the tumor culture, 4 cc. of a mixture of equal parts of treated filtrate and of culture fluid, and finally, 4 cc. of a mixture of equal parts of treated filtrate and of the supernatant fluid of an embryonic culture. The injections were made in the same order, starting at a different place in each chicken. The positive experiment (No. 4 of this series) gave the following results: The untreated sand filtrate produced tumors in doses of 0.2 to 1 cc. 5 cc. of the chloroformed filtrate, and 5 cc. of the fluid of the tumor cultures were inactive in all chickens. The mixture of treated filtrate and culture fluid from the chicken tumor produced a typical sarcoma in the right breast of chicken 1, and in the right wing of chicken 3. The mixture of treated filtrate and of fluid from the embryonic culture was negative in chickens 1 and 2, but produced a rapidly growing tumor in the left wing of chicken 3. The two tumors of chicken 3 were noticeable after 2 weeks, and killed the animal after 3 weeks. Chicken 1 developed a large tumor and died after 5 weeks.

DISCUSSION.

Obviously Gye's reactivation experiment is very hard to duplicate and works only under very strict experimental conditions. The cause for this is undoubtedly the difficulty with which the proper degree of chloroformation is obtained. The margin between incomplete inactivation and irreversible inactivation due to an excess of chloroform action seems to be very narrow. It was thought safer to adjust conditions in such a way that they were close to an irreversible inactivation of the tumor producing agent rather than to have incomplete inactivation. This may account for the many negative experiments that we obtained. On the other hand, it is evident from our positive experiment that the reactivation is an unspecific process, since an embryonic culture proved just as effective in this respect as a tumor culture, yet it is a postulate of Gye's theory of the origin of cancer that the power to reactivate be confined exclusively to malignant tissue. The reactivation of the chloroformed filtrate can, therefore, not be regarded as proof that a culture of tumor tissue contains a living organism.

SUMMARY.

The supernatant fluid from a culture of embryonic tissue was just as effective in reactivating a chloroformed extract from a fresh tumor as was the supernatant fluid from a tumor culture. Due to the unspecific nature of the reactivation process, Gye's interpretation that a living organism is involved in this type of experiment, is not warranted.

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[1] Gye, W. E., *The Lancet*, 1925, ccix, 109.

Pacific Coast Branch

Mills College, Oakland, Calif., June 17, 1926.

3232

The Interaction of Genes in Development.

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Since it has been shown that the transmission of most hereditary traits can best be explained on the theory of the gene, it is important to try to trace the relation between particular genes in the fertilized egg and the correlated somatic manifestations that appear during development. That such correlations do exist has been abundantly demonstrated, but just how the gene exerts its effect on the soma is still unknown. As a first step towards an analysis of this problem one may inquire into the interrelation between the genes themselves. Here there are two possibilities to be considered: (1) that the genes interact with each other to give the cell its structural and functional characteristics; (2) that each individual gene separately exerts its own specific influence.

These alternatives may be illustrated by the following schemata, in which A and B represent the genes in a cell whose contribution to the soma may be either S or S' (depending on such factors

$$(1)\quad \left.\begin{matrix}A\\ \\ \mathrm{B}\end{matrix}\right\}\text{—}N\text{—}\left\{\begin{matrix}S\\ \\ S'\end{matrix}\right. \qquad\qquad (2)\quad \begin{matrix}A\text{—}n\text{ —}S\\ \quad >n'\text{—}S'\\ B\text{—}n''\quad\end{matrix}$$

as topographical relations, stimuli, etc.). In (1) all the interaction between A and B is in the nucleus and N is the single resultant effect—a particular type of protoplasm. This would seem to correspond to a common conception among experimental biologists. In (2) A and B are more or less independent, n, n' and n'' representing distinct elements in the total of cytoplasmic potentialities. In this case it is not the genes themselves, but certain of their secondary products that interact, so that while A ultimately affects both S and S', B affects only one of them. The determination of which of these alternatives is in accord with the true situation is of considerable importance to the understanding of protoplasmic reactions in general.

Data bearing on this question have been obtained from experiments with the Y gene in mice. Individuals with the genetic formula $CCSSYy$ show two very different traits; they have yellow fur and they become decidedly adipose after reaching maturity. When SS (self color) is replaced by ss (spotting) the amount of yellow in the pelage is greatly restricted, and it might be expected, if the interaction were according to the first scheme, that the effect of Y on adiposity would also be limited. But a considerable series of controlled observations has shown that mice of the genetic constitution $ssYy$ become fully as adipose as those that are $SSYy$ and greatly exceed those that are $ssyy$ or $SSyy$. This may be explained on the basis of the second scheme if Yy and ss be substituted for A and B respectively. S in the schema now represents adiposity, S' the amount of yellow fur. In this connection it might be objected that spotting is not a modifier of yellow in the usual sense of the term. However, there is some evidence that it really is such, and if it were not, it would still furnish evidence of another kind in favor of the second alternative.

Likewise, when CC (color) is replaced by cc (albinism) yellow is entirely suppressed but the adiposity is uninfluenced as determined by tests in which, as before, litter mates were used for controls. The following table shows the genetic formula, the color, and the weight at seven months of three pairs of littermate sisters.

TABLE I.

Genetic formula	Color of fur	Weight at seven months
$CCS\text{-}Yy$	Pure yellow	44 grams
$CCS\text{-}yy$	Pure black	24 ”
$CCssYy$	Spotted yellow	54 ”
$CCssyy$	Spotted brown	35 ”
$ccSSYy$	Pure white	52 ”
$ccSSyy$	Pure white	24 ”

A further effect of the *Y* gene is that it acts as a lethal when homozygous. Consequently a series of albino mice carrying *Y* were tested to see if a combination of genes which suppresses its effect on color would also suppress its lethal action. Among the forty-four individuals tested none was found in which *YY* had failed to act as a lethal, which indicates that *cc* had been without influence in this respect. The chances against the observations being purely fortuitous are about thirty thousand to one.

The three known effects of the *Y* gene occur at about six, twenty-five, and ninety days after fertilization of the egg, and the evidence cited indicates that a complex which modifies one of these effects may have no necessary influence on either of the others. This points to the conclusion that the genes do not mutually interact in the beginning to produce a certain individual type of protoplasm, but that, on the contrary, they behave as separate dynamic factors in development.

3233

Correlation of Protozoan Infections of Human Mouth with Extent of Certain Lesions in Pyorrhea Alveolaris.

H. CORWIN HINSHAW. (Introduced by C. A. Kofoid.)

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Gingival exudate or interproximal debris has been obtained from 350 individuals, displaying a wide variety of peridental conditions, and has been cultivated for 48 hours at 37° C. in Boeck's medium. This technique reveals many infections of *Endamoeba gingivalis* and *Trichomonas buccalis* in the gums that are negative to other means of diagnosis.

In a quantitative survey of 100 dental students in the University of California we have found that infection with *Endamoeba gingivalis* is closely correlated with the extent of gingival pocket formation (Table I).

TABLE I.
Relation of amoebic infection to depth of gingival pockets.

Maximum depth of pockets	0-0.5 mm.	0.5-1.0 mm.	1.0-2.0 mm.	Over 2.0 mm.
No. infected with *E. gingivalis*	1	6	4	9
Percentage of infection	2.27	15.0	57.14	100.
No. free from protozoan infection	43	34	3	0
Percentage not infected	97.73	85.0	42.86	0.

We are indebted to Dr. F. V. Simonton for his generous aid in examining this group.

One hundred and eighty-six prisoners at San Quentin have been cultured and superficially examined. *Trichomonas buccalis* is recovered in culture quite commonly from advanced cases of pyorrhea. This organism is seldom found in smear preparations. It is never found in the normal mouth but may be found in cases with a recent history of active pyorrhea. (Table II.)

TABLE II.
Incidence of protozoan infections of the mouth in San Quentin Prison.

Superficial diagnosis	Advanced pyorrhea	Uncertain and anomalous cases	Normal mouths and incipient pyorrhea
No. infected with both *T. buccalis* and *E. gingivalis*	43	9	0
No. infected with *T. buccalis* only	0	5	0
No. infected with *E. gingivalis* only	6	42	18
No. negative for protozoa	0	10	53

Trichomonas buccalis is definitely antagonistic to *E. gingivalis in vitro,* so cases harboring both parasites may yield a falsely negative report for the latter when the culture method alone is employed. Putrefactive proteolytic bacteria in excess are antagonistic to the development of buccal protozoa in culture. Protozoa are sometimes closely restricted to limited areas in the gums.

Sixty-four cases have been cultured without adequate dental examination. Twenty-four (37.5 per cent) of these were infected with *E. gingivalis*. Three (4.68 per cent) were infected with *T. buccalis*. The remainder were free from protozoan infections in the mouth.

From these data we conclude that protozoan parasites do not occur in the normal mouth. *Endamoeba gingivalis* occurs in most, if not all, cases of incipient to advanced pyorrhea. *Trichomonas buccalis* appears frequently in cases of advanced pyorrhea, or occasionally in cases with a history of effectively treated pyorrhea.

3234

A Preliminary Study of Rhodobacillus Palustris, Molisch.

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L. B. BECKING.

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The form studied was obtained by Mr. W. Meehan from the intestines of a Cerambycid beetle. It proved to be a true purple bacterium with the characteristics of Molisch's *Rhodobacillus palustris.*[1]

The Molisch group of the purple bacteria was omitted in a previous study by one of us. Here the spectra of various purple bacteria were considered, including the sulphur bacteria, brine bacteria and the lichen symbiont *Chiodecton.*[2] It seemed, therefore, desirable to determine the spectrum characteristics of the *Rhodobacillus.*

Microspectra were taken with the microspectrograph[3] on Wratten panchromatic film of a very thin layer of pure culture bacteria (one bacillus thick). A control photograph of the lamp was taken on the same film.

The negatives were measured out by means of densitometer attachment to the radiomicrometer.[4] First the raw data were used to calculate the position of the absorption bands. It was found that *Rhodobacillus* is a true purple, inasmuch as the position of the bands agrees closely with the data already obtained (Becking 1. c.). The extrapletions were carried out by means of a triple set of assumed wave lengths and the prismatic spectrum converted by means of v. Hartmann's formula.

Calculated for Purple Bacteria	Found for Rhodobacillus
590μμ	590μμ
568	absent
548	548
515	512
500	500
480	480
459	458
446	443
438	430?

By the use of Schwartzschild's law the per cent absorption for various wave lengths was determined by means of comparison with the lamp negative. The following table shows the absorption for various wave lengths:

Wave length	Per cent absorption
590μμ	53.5
580	45.0
568	54.7
560	50.7
548	55.7
530	33.7
520	42.8
458	36.8
430	60.8
410	61.5
390	74.5

Integration of the plotted absorption curve gives an absorption between 590μμ and 410μμ of 45.8 per cent for the single bacterium.

The absorption characteristics of a somewhat thicker layer of *Rhodobacillus* was ascertained by means of a radiomicrometer and glass screens. The light-green "Corning" screen transmitted only visible light between 680 and 400μμ. The other screen was a carbon glass which transmitted only infra red up to the visible red. The lamp used was an Edison Platinum filament lamp, run on a transformer.

Light source	Per cent absorption
Pt. lamp, no screens	36.6
Pt. lamp, visible only	73.2
Pt. lamp, infra red only	19.7

This tends to show that the infra red absorption bands of the purple bacteria, as described by Buder,[5] do not have much influence on the absorption curve.

Another culture of apparently the same species of *Rhodobacillus* was isolated from corn starch paste. The identity of the two forms was established by routine bacteriological procedure. As Molisch' characterization is inadequate, we will attempt to give a short diagnosis of the form.

Short stout rods, 2-3μ long. Gram negative, non-motile, no endospores. Colonies on gelatin; rapid liquifier, colonies sunken, salmon pink, coalesce after a few days. Gelatin stab; rapid liquefaction, pigment formation at the top. Agar colonies; small, even-edged, slightly raised above the surface. The color is denser in the center of the colony. Agar stab; good growth, facultative anaerobe. Lead acetate stab, slight H_2S formation. Broth; not very strong growth, no pellicle formed, no pigmentation, except after standing for 5 to 7 days at 20° C.

Litmus milk becomes slightly acid but does not curdle or peptonize. No indol formation after 2 days at 37.5° C. Nitrates are reduced to nitrites.

Pigment formation takes place in light or dark, even at 4° C. At 37.5° C. no pigment is formed, and growth is only moderate. The pigment is first salmon pink. Later (due to the increase in bacteriochlorin) the color becomes dark crimson red. After a few weeks on agar the color begins to fade out.

Rhodobacillus does not form gas on any carbohydrate media investigated. It formed acid from the following substances: dextrose, maltose and mannitol. No acid was formed in lactose, galactose, sucrose and levulose, although these sugars seemed to form an adequate source of carbon for the bacteria.

According to the modern bacteriological nomenclature[6] the form studied belongs to the genus *Serratia*. The general bacteriological characteristics of the *Chromobacteria* leave much to be desired.

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[1] Molisch, H., *die Purpurbakterien*, Jena, 1907, page 14, plate I, figs. 1-4.

[2] Becking, L. B., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 523.

[3] Becking, L. B., and Ross, P. A., *J. Gen. Physiol.*, 1925, ix, 3.

[4] Field, J., and Becking, L. B., *J. Gen. Physiol.*, 1926, ix, 445.

[5] Buder, J., *Jahrb. Wiss. Bot.*, 1919, lviii, 525.

[6] Bergey, H., *Manual of determinative Bacteriology*, 1923, 85.

3235

The Examination of Guinea Pig Dental Enamel Matrix by Celloidin Section.

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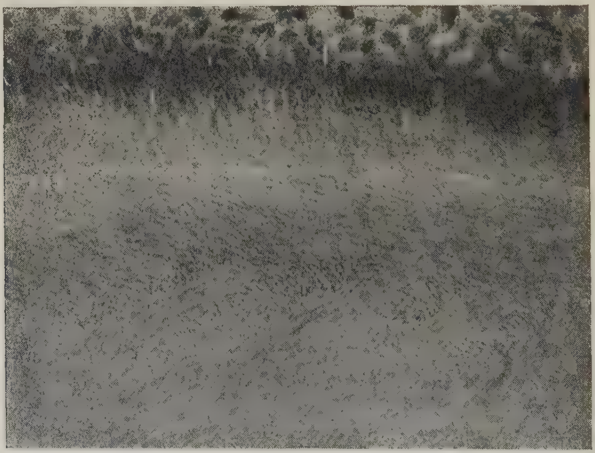
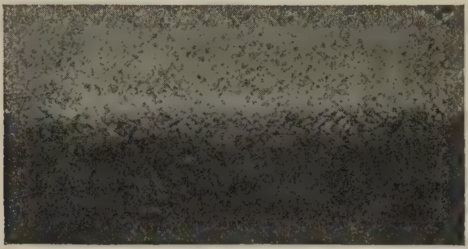
Much discussion has taken place regarding the organic matter found in dental enamel. Certain workers[1] have endeavored to show that calcification in this structure is so complete that no organic matrix remains. Williams[2] could not find any material in enamel which is stainable. Evans,[3] however, has shown that the organic matter in this tissue lies between one and two per cent in the human tooth. Bödecker and Gies[4] lately have demonstrated the presence of protein within the enamel by microchemical methods. That sections may be prepared showing certain points of morphology of this small amount of organic material within enamel has been stated recently.[5]

Celloidin sections of enamel matrix of the tooth of the guinea pig may be prepared. Methods have been elaborated by one of us (A. W.) whereby decalcification may be carried on through a supporting structure of celloidin placed about the tooth. This proced-

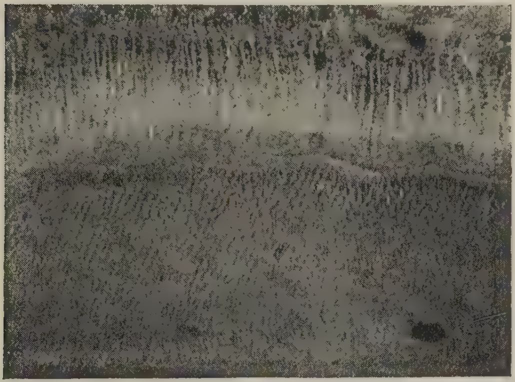


Enamel Matrix in Contact with Ameloblastic Tissue in Guinea Pig Incisor Tooth. This shows the Tendency of Enamel Rods to Form Spirals. Celloidin Section.

ure will be described at a future time. It is somewhat different from that described briefly by Bödecker and Gies.[4] We have worked with that portion of the incisor lying midway between the gingival edge and the apex. This is moderately young enamel, therefore. Transverse sections may be prepared showing ameloblasts, enamel, dentine and odontoblasts in proper relationship.



Intermingling of Dentinal Tubules with Enamel Rods in Matrix of Guinea Pig Incisor Tooth. Celloidin Section.



The Continuity of Enamel Rods with Ameloblasts in the Matrix of the Guinea Pig Incisor Tooth. Celloidin Section.

Examination of sections prepared thus contributes certain information to our conception of the structure of enamel. Nearly all our knowledge thus far has been based upon work done with ground sections. In the guinea pig, there is definite articulation of the enamel matrix with the ameloblasts through the region of Tomes process. The enamel rods are projections from the individual ameloblastic cells. Thus we find ourselves in conformity with the belief of Mummery.[6]

Careful decalcification shows a honeycomb structure of the enamel matrix. The prisms of the enamel rods upon solution by acid leave a series of tubes which in these preparations are nearly circular in cross section. Similar structure in human enamel has been revealed by Malleson.[5] This observation appears to make untenable the position of Carter[7] in application to the guinea pig. He has stated that there is no tubular matrix. Our preparations show the tubes in the matrix lying in whorls which are made up of spirals. Although there may be some distortion here as the result of treatment with reagents, this spiral arrangement is not entirely artifactual.

In the guinea pig anastomosis or branching of enamel rods is seen occasionally. There is much decussation also. The contour of the tooth suggests the possibility of branching, but heretofore ground sections have not been decisive in results dealing with this question.

The enamel rods and the dentinal tubules intermingle in the guinea pig for a short distance. However, the tendency is rather for the dentinal tubules to penetrate the zone of the enamel rather than for the reverse condition to occur. We have not been able to indicate a direct continuity between these two tubular structures. This fact does suggest though the possibility of passage of small amounts of nutrient material from the dentinal tubules to the enamel.

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3236

Identification of Skin-reacting Substance in Blood Sera from Tuberculous Patients and Animals.

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A biologic method has been devised for identifying a specific skin-reacting substance in the blood serum of tuberculous patients and animals. The point of departure was an earlier observation reported elsewhere[1] that sensitization in normal guinea pigs could be accomplished with fractional tuberculins prepared from non-protein substrates.

Duplicate series of healthy white guinea pigs, weighing 450 to 500 grams, were used in each set of experiments. These were grouped as follows:

(1) Normal guinea pigs previously injected with tuberculin fractions.

(2) Normal, untreated guinea pigs, and

(3) Tuberculous guinea pigs with early, moderately advanced, and far advanced infections.

The animals were tested with (a) human sera from early and far advanced tuberculosis, (b) same sera heated at 60 to 65° C. on a water-bath for 45 minutes, (c) normal human serum, (d) guinea pig serum from early and moribund stages of tuberculosis. Intracutaneous injections of 0.02 cc. of each serum were given for each test. The guinea pigs of the first series were injected subcutaneously with increasing doses of one of the fractional tuberculins over a period of 7 weeks, receiving a total amount of 2.2 cc. of each substance. Ten to 14 days after the last injection, intracutaneous tests were applied with the materials described. Reactions were recorded after 24, 48, and 72 hours, and observations made until the sites of injection appeared normal.

Groups of normal, sensitized, and tuberculous guinea pigs react differently toward intracutaneous injections of minute amounts of blood serum from tuberculous patients or animals. The specific skin reactions appear to depend upon the stage of infection to which the serum corresponds. Observed differences are referable to an early or far advanced tuberculous process, and if this has been arrested, to the presence or absence of toxemia. This relationship of serum and its source is influenced, moreover, by the stage of tuberculosis in the test animal. Furthermore, heating tuberculous serum at a temperature of 60 to 65° C. reduces a skin reaction markedly or extinguishes it completely when all other conditions are kept constant. Normal guinea pigs, for example, react positively toward tuberculous serum but do not give a reaction with the same serum heated. Similar effects are noted in the group of animals sensitized by repeated injections of tuberculin fractions, and also in the groups of tuberculous animals. Sera from tuberculous guinea pigs in a moribund state give a delayed and transitory skin reaction or a negative one in animals ordinarily responding in varying degree to sera from early or advanced tuberculosis. Finally, normal human or guinea pig serum fails to elicit any reaction in the different groups of animals. As evidence of a more general tissue response in reacting guinea pigs, there is a pronounced inguinal adenopathy.

The mechanism of the reactions can be explained on the basis of addition or subtraction of one or more interacting substances in tuberculous sera and in the tissues of the experimental animal. For example, a group of normal tuberculin-sensitized guinea pigs reacts more violently and persistently to intracutaneous injections of serum from advanced toxemic tuberculosis than to serum from an early, non-toxic, arrested infection. This group of animals is strongly endowed with tuberculin-reacting elements and with antibodies for products of tubercle bacilli. Injection of the serum causes an augmented reaction by introducing appreciable amounts of toxin. In the same group, however, the results are negative when heated serum is used, indicating that toxin is not introduced. In the untreated normal guinea pigs, the same holds true because of the interaction of toxin and normal antibodies.

A heat-sensitive skin-reacting substance of the nature of a toxin has been identified in the blood serum of tuberculous patients and guinea pigs. It is not found in normal human or guinea pig serum. The substance does not behave like tuberculin or its related elements which are heat-resistant, and exists independently of these. It is present in largest amounts in far advanced tuberculous infections with profound toxemia, and is apparently destroyed by heating at 60 to 65° C. for 20 to 45 minutes. In normal animals this unheated substance gives a positive skin test owing to its interaction with normal antibodies for tubercle products.

The method devised for identifying this specific skin-reacting substance depends, in principle, upon a living "indicator", the guinea pig, which measures related or identical elements in the serum to be tested. The procedure was made possible by a previous demonstration that normal animals could be sensitized with fractional tuberculins prepared from non-protein substrates.

Skin reactions obtained according to the technic described in these experiments are referable to an addition or subtraction of one or more of the interacting substances present in tuberculous serum and in the tissues of the experimental animal.

Evidence is adduced to show that the heat-labile substance, probably a toxin, in tuberculous sera, can be used as a measure of the circulating antibodies in normal and tuberculous persons. A test of this type may serve as an index of the bodily resistance to tuberculous infection in both groups.

Certain theories and hypotheses regarding tuberculin and skin sensitiveness find controllable experimental evidence in the observations described. An explanation is offered for negative tuberculin tests in far advanced tuberculosis, and furthermore, positive reactions with tuberculous sera in apparently normal persons are to be attributed to a toxin. Accordingly, the results with the autoserum test of Lenz[2] and modified autoserum[3] and Wildbolz reactions[4] take on a somewhat different interpretation.

Studies are now in progress to determine the value of a test which is fundamentally like the Schick test in diphtheria, and the Dick test for scarlet fever. Already experimental evidence is at hand indicating the presence of a toxic substance in filtrates obtained from the growth of tubercle bacilli in special culture media.

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3237

Newer Concepts as to the Origin and Nature of Antibodies.

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Practically the only complete and consistent theory as to the origin and nature of immunological antibodies is the theory based on the specific receptor hypothesis. Physiological data collected in our laboratory during recent years are sufficient to my mind to prove this theory wholly untenable. I believe the wide acceptance of the specific receptor hypothesis by clinicians and practical serologists constitutes today our most serious handicap to immunological progress, and that the theory should be replaced by concepts more nearly in accord with accepted facts of biochemistry. The following are some of our typical findings:

(a) The assumed multiplication of sessile receptors in hypersensitive and immune tissues should increase the specific affinity of these tissues for soluble antigens. Yet, in our hands, perfusions of isolated blood-free organs of normal, hypersensitive and immune animals with soluble antigens have shown no demonstrable differences in their specific tissue-antigen affinities.[1]

(b) The blood-free tissues of immune animals should be specifically hypersensitive, due to an increased number of sessile receptors. Yet the isolated blood-free organs of immune dogs show no suggestion whatsoever of specific hypersensitiveness.[2]

(c) The blood of immune animals should serve as a portal defense to the fixed tissues by shunting the injected specific antigen from the fixed tissues. Yet a hypersensitive dog, three-quarters of whose blood had been replaced by transfusion from an immune donor, is typically hypersensitive on immediate physiological test.

(d) Circulating antibodies should be *qualitatively* identical at all stages of sensitization and immunization with the same antigen. Yet the blood of a hypersensitive dog will invariably render a normal dog passively hypersensitive, while no demonstrable hypersensitiveness is transferred to a normal dog by fractional transfusion from an immune donor.[3]

It would be comparatively easy to replace the discarded specific receptor hypothesis with a theory based on the assumption that fixed and circulating intibodies are slowly formed in the body as a result of the interplay between injected antigens and extracellular and intracellular, hydrolyzing and synthesizing enzymes, and by these enzymes specifically adapted to the antigens or to the primary or secondary products of antigen hydrolysis.

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Missouri Branch

St. Louis University School of Medicine, St. Louis, Mo.,
May 26, 1926.

3238

Rosette and Granule-Hyaline Phagocytes in Tissue Cultures Stained Supravitally with Neutral Red.

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The rosette and the diffuse granule types of phagocytes were observed by Sabin, Doan and Cunningham[1] in peritoneal exudates stained supravitally with neutral red. The latter type may contain few dye granules or none whatever,[2, 3] and is therefore called the diffuse granule-hyaline type. There is no proof that either of these phagocytes is related to the peroxydase-reacting monocyte of Naegeli.[4] In the human blood there is some evidence that the Naegeli monocyte is neither the rosette nor the diffuse granule-hyaline cell.[3] In the human, the epithelioid cells of the tubercle of lymph glands contain the largest and most characteristic rosettes seen, but these cells in smears do not react to the peroxydase test that colors intensely the Naegeli monocytes in the blood smears of the same individual.[6] The final conclusion of Cunningham, Sabin and Doan[5] is that the rosette type arises from the reticulum, and that the diffuse granule cell comes from endothelium. These investigators did not find the rosette cell in the lymph nodes. In my experiments[2, 3] the rosette cell was found to be abundant in the lymph nodes where it was identified as the reticulo-endothelial cell. In tissue cultures of rabbit lymph nodes the rosette cell is almost the sole phagocyte present. On the other hand, the phagocytes of the tissue cultures of rabbit liver are almost exclusively of the diffuse granule-hyaline type.[6] On the basis of these findings, considered together with the other data set forth in the papers cited, the conclusions reached were that the rosette cell arose from the reticulo-endothelium of lymphoid tissue and that the diffuse granule-hyaline phagocyte arose from the vascular endothelium.

Tissue cultures of rabbit spleen were made in order to determine the occurrence there of the two types of nonoxydase-reacting phagocytes. The methods used have been described elsewhere.[6] Upon supravital staining on the fourth to the seventh day the explanted bit of tissue often separates from the remainder of the plasma clot, and onto the glass beneath the phagocytes are spread out where they can be photographed to advantage. To preserve the dye in the cells the preparations are fixed for one hour and Zenker-formol solution, washed quickly, bottled dry, allowed to dry for one hour and mounted in balsam after clearing in xylol. Most of the phagocytes are of the rosette type. Extremely long cytoplasmic processes which approach reticulum fibers in size often connect the cells. In their reaction to neutral red and in structure they are like the reticulo-endothelial cells of the rabbit lymph node cultures. The occurrence in the spleen cultures of so many rosette cells that have all the characters of those present in lymph node cultures leads one to think that there is present in the spleen fixed tissue of the same type as the reticulo-endothelium of the lymph nodes.

The diffuse granule-hyaline cells are present in all cultures and small groups of them connected by cytoplasmic processes may be found. After placing the cultures in the neutral red solution, dye granules are often seen first in a focus near the nucleus, but later they appear scattered about elsewhere in the cytoplasm. In their reaction to neutral red there is much similarity between fibroblasts and diffuse granule-hyaline phagocytes. Therefore, the intravenous injection of carbon suspension some time before removing the spleen for the cultures marks the phagocytes and facilitates the accurate differentiation of the diffuse granule-hyaline cells and fibroblasts. The diffuse granule-hyaline phagocytes are like those of the rabbit liver cultures.

CONCLUSIONS.

1. In tissue cultures of rabbit spleen there are present the two varieties of mononuclear phagocytes which were demonstrated by Sabin, Doan and Cunningham in material obtained directly from the spleen of the rabbit under anesthesia. Both cells grow as reticular tissue with cytoplasmic processes which connect the individual phagocytes.
2. Since the rosette variety of phagocyte is constantly present in the tissue cultures of rabbit spleen in such large numbers, lymphoid reticulo-endothelium of the type found in lymph nodes is thought to be present in the normal spleen.

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3239

Reaction Produced by Lipoid Solvents in Animals Fed Diets Deficient and High in Vitamin A.

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(Introduced by M. T. Burrows.)

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Lipoid solvents introduced into the subcutaneous tissue of rats fed a diet deficient in vitamin A remain well encapsulated by a zone of fixed tissue cells. This zone of tissue undergoes hyalinization after a period of thirty days and the mass of lipoid solvent remains encapsulated and quiescent within this zone.[1] The livers removed from these animals present a normal appearance grossly and microscopically.

The lipoid solvents introduced into the subcutaneous tissue of rats fed on a diet rich in vitamin A break up into numerous smaller droplets, each of which is surrounded by actively dividing fixed tissue cells. After a period of sixty to ninety days nests of actively growing cells are dispersed among the remainder of the tissue in this zone, which has undergone hyalinization during this period.[1] The livers removed from these animals present a brownish-yellow appearance in the gross. Microscopically, the liver parenchyma shows a diffuse and general infiltration of fat droplets.

Lipoid solvents are encapsulated by a similar zone of fixed tissue cells in animals fed a balanced dietary.[2] In a few cases we have noted a partial breaking up of the solvent around its periphery. The liver is normal in appearance.

These lipoid solvents absorb the ergusia of the cell. Vitamin A is or furnishes the ergusia. If vitamin A is withdrawn from the system, the lipoid solvent becomes encapsulated in the subcutaneous tissue and remains quiescent. The tissue around the solvent undergoes hyalinization due to the absorption of the ergusia by the lipoid solvent.

When vitamin A is added to the system the lipoid solvent remains active for a long period of time. The lipoid solvent absorbs ergusia until it is saturated, then breaks up into droplets and migrates to other organs in the body. A fatty infiltration of the liver occurs under these conditions.

There is no evidence that part of the whole mass of lipoid solvent enters the general circulation and produces the change in the liver, but probably it is the more mobile portions or more volatile fractions dissolved in them. Coal tar, washed with ether and heated, does not produce this change. An early and complete hyalinization takes place about drops of this substance introduced into the subcutaneous tissue. It absorbs all the ergusia from the cells with which it comes in contact. This indicates that it is the light oil in the coal tar that causes the tissue change as noted above.

Lipoid solvents treated with ultra-violet light, mixed with extracts of old cultures of *B. tumefaciens* or the Allen-Doisy hormone produce the same changes in the tissue about the drops, excepting that the cells do not degenerate so readily and they show much more proliferation.

That lipoid solvents cause anaemia and fatty infiltration of the liver is well known. Benzol is in this class. It has been possible for us to show that such substances do not act or cannot be transported so they can act except when the system is saturated with vitamin A.

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3240

The Effect of Vitamin Feeding on the Growth of Cancer.

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Cramer[1] states that the removal of one or the other of the vitamins from the diet of animals with transplanted and growing tumors has little or no effect on the growth of the tumors. The animal alone suffers from the deficiency. We have noted a similar result excepting when very large doses of vitamin B are given in a diet deficient in vitamin A. Rats inoculated with the Jensen sarcoma were used for these experiments. Rats given very large doses of vitamin B in a diet deficient in vitamin A suffered a marked anemia with a rapid disappearance of the tumor in a few cases and an early death of the rats in most cases. Tumors in two men broke down with this diet much the same as with X-rays. The tumors were lip cancers metastasizing to the neck. These patients also suffered marked anemia and it was necessary to stop the treatment after a short time, not only on account of the anemia, but because of a developing parenchymatous nephritis.

Quite different from the animals, men with malignant tumors have responded more readily to small changes in the vitamin A content of the diet. A man with a melanoma arising from a birthmark on his head and a large mass of metastases on one side of his neck, recovered from his cachexia and anemia completely after six weeks, and worked hard during the next four months, maintaining his nutrition and suffering no extension of his tumor. The diet he received contained a large amount of vitamin A and a moderate amount of vitamin B. He left us at this time and a competent diagnostician thought the tumor was not malignant on account of the patient's good physical condition. He removed the tumor with eventual disaster. Another case, a girl of 27, with thousands of metastases over her body, recovered from her cachexia completely and the tumors remained stationary for over three months, when she died of intestinal perforation. A single very large dose of vitamin B caused a marked loss of vigor and increased her measurable tumors one-half, and on another occasion a small dose of X-ray to a painful area of her back also increased the size of her skin metastases. These tumors regressed again on a diet high in vitamin A. X-rays, as we have shown, liberates vitamin B in the organism.

Even old men, having a marked arteriosclerosis and myocarditis, besides cancers of mouth and neck, have recovered from their cachexia and have responded much better to X-ray treatment under a high vitamin A diet than usual.

As pointed out in other papers,[2] our experiments have shown that the stimulus for the growth of body cells is a water soluble and actively diffusible substance, which has been called the archusia. It is formed by the cells but cannot be retained by them excepting in a restricted stagnant environment. This substance is not specific in many instances at least, and when fed to animals has been found to be vitamin B.[3] The normal animal organism contains not only this growth stimulus, but an excess of growth inhibitor, a lipoid substance known as the ergusia. The ergusia is formed by all cells in nature but not in quantities sufficient to inhibit their growth. It is a necessary and intimate part of their protoplasm. The ergusia is either vitamin A or formed from vitamin A.[4] It is forced in excess on the cells of higher animals from the food. In the presence of this high ergusia or vitamin A the cells do not form and retain ample archusia or vitamin B for their life. This must be supplied also from the outside. In low concentration (S^1) the archusia (S) or vitamin B has no effect. In medium concentrations (S^2) it causes the cells to differentiate and function. In high concentrations (S^3) it causes them to lose their differentiation and grow. In all higher concentrations (S^4) they degenerate.

The ergusia is used for differentiation. The proper life of the organism depends on the proper balance of these two vitamins. Cancer develops in the organism at any point where the cells are relieved of the excess of vitamin A and are crowded into masses having a poor blood circulation where they can form and retain ample archusia (S) for their independent growth.

Any slight addition of vitamin A to such a system must retard its growth. Any slight addition of vitamin B must accelerate this growth. The addition of greater amounts of vitamin B must disturb function as well as destroy a cancerous tissue. Cachexia results from either removing the vitamin A from the organism or increasing the vitamin B. Vitamin B is liberated by all actively growing cells. Cancerous tissues liberate large quantities of it. The same large quantities are liberated by actively growing bacteria. A patient with a moderate tuberculous lesion grows excessively. With a larger lesion he suffers cachexia unless given a diet rich in vitamin A. He will suffer the cachexia from a much smaller lesion if this vitamin is withheld. The same has been found to be true for cancer patients. A patient suffering from the terminal fever or *spleno-myologenous leukemia* was relieved of his fever for a period of ninety days with a diet rich in vitamin A. His fever could be returned by a single large dose of either vitamin B or X-rays. He was able to be up and around, dying finally of a hemorrhage into a leukemic spot in his pons. He might have been relieved of this at an earlier time if proper local X-ray treatment had been given.

It has thus been possible in our experiments to show that the symptom complex of many diseases can be reduced to terms of vitamin imbalances. The growth of the cells in cancer is not the result of external agents, but the result of a previous disturbance in the balance of vitamins at a local point in the organism. It is the high vitamin A content of the tissues which prevents the cells of the body from growing independently at all times. Cancer is induced by anything which removes this excess from a locality or increases the vitamin B in that locality. Its subsequent effect on the body is the result of its liberating an excess of vitamin B to other tissues and the removal of vitamin A used for the growth of its cells.

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3241

The Lactic Acid Content of Cerebrospinal Fluid.

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The lactic acid content of a series of normal and pathological specimens of spinal fluid was investigated, and in a number of cases compared with the blood lactic acid of the same patient after one-half hour of absolute rest following the lumbar puncture. Analyses were made by the sulphuric acid method of Clausen.[1]

A spinal fluid was considered normal if it was clear and colorless, under normal pressure, had 10 or less cells per cm., gave a Pandy test plus one or less, a negative Wasserman and a Lange curve not rising over 2. Of 13 spinal fluids which could be considered normal, using this standard, the lactic acid content varied from 11 to 27 mg. per 100 cc., with an average of 19 mg. In 9 cases in which the lactic acid of the blood was also examined, the value for lactic acid in the spinal fluid was found to be 60 to 100 per cent of that in the blood. The average was 75 per cent. Killian[2] reported a lactic acid content of 8 to 15 mg. per 100 cc. in 5 normal cases and in 15 miscellaneous cases found a percentage ratio of spinal fluid lactic acid to blood lactic acid of 80 to 90 per cent. In our series a number of cases of syphilis of the central nervous system, of old hemiplegia, of epilepsy (between convulsions), and of amyotrophic lateral sclerosis, the values for lactic acid in the spinal fluid were within normal limits. In 2 cases of acute meningitis the lactic acid content was found to be greatly increased. A case of influenzal meningitis had a lactic acid content of 91 mg. per cent and one case of *Streptococcus meningitis* 150 mg. per cent. Blood lactic acid studies could not be made in these cases. In a case of xanthochromia, caused by trauma to the spinal meninges and of four days duration, the lactic acid content was 35 mg. per cent and the percentage ratio to the blood lactic acid was 140. In a case of brain abscess the spinal fluid lactic acid was 46 mg. per cent.

The most interesting specimen was from a patient with a probable spongioblastoma of the medulla (diagnosis confirmed by operation) who underwent a spinal tap before the diagnosis of brain tumor was made. The spinal fluid lactic acid was 24 mg. per cent, well within the normal limits, but the blood lactic acid was only 10 mg. per cent, giving a percentage ratio of spinal fluid lactic acid to blood lactic acid of 240. We also studied another patient who undoubtedly has a brain tumor, though none was found on exploratory craniotomy. The ventricular fluid of this patient contained 41 mg. per cent of lactic acid, and the blood lactic acid just before operation (which was done under local anesthesia) was 20 mg. per cent, giving a percentage ratio of 205. The increased lactic acid in the fluid of these cases may perhaps be explained by the observations of Warburg and Minami[3] on the large amounts of lactic acid produced by tumor cells. A number of additional cases of brain tumor were studied in which the lactic acid content of the ventricular fluid was normal. In these cases, however, as demonstrated by operation, no surface of the tumor was in contact with the fluid from which a specimen for analysis was obtained.

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3242

Effect of Drugs Upon Tonus Waves in Excised Auricle and Coronary Vessels of Terrapin.

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Excised terrapin auricles show continuous tonus waves 20 to 42 hours after excision, when kept in oxygenated Ringer's solution pH 7.8 to 8.0, at room temperature 20.5 to 27° C. Immediately after excision, decreasing the pH from 7.4 to 7.0 causes a disappearance of the tonus waves.[1] These waves reappear when the auricles are replaced in a solution of pH 7.4. Eighteen to 42 hours after excision these same auricles fail to respond by disappearance of tonus waves until a pH of about 6.2 to 6.0 is reached. In one case the tonus waves disappeared only after the pH was reduced to 5.8 and showed optimum tonus waves at pH 6.8. Phosphate buffered solutions tend to stimulate tonus waves. In some auricles, in which carbonate-HCl buffered Ringer's solution pH 6.6 caused the tonus waves to disappear, phosphate buffered Ringer's solution 6.2 caused an increase in tonus waves, and when this solution was replaced by Ringer's pH 7.4 temporary disappearance of the tonus waves occurred.

Small doses of adrenalin (alkaloid) 1:1,000,000,000 solution cause increased tonus in the fresh preparation[2] (stimulation of the positive tono-trophic fibres) but not in the 24-hour strip. The addition of adrenalin to the bath does not initiate these tonus waves in auricles in which they are not present. Larger doses of adrenalin (alkaloid) 1:100,000,000, cause a disappearance of the tonus waves (stimulation of the negative tono-trophic fibres).[2, 3] This decrease in tonus is independent of changes in pH of the bath. Tyramine acid phosphate influences tonus waves in the same manner as does adrenalin, but a higher concentration is required to stimulate the negative tono-trophic fibres.

Pure pituitary extract,[4] histamine phosphate (British Drug Houses), ergamine acid phosphate (B. W. & Co.), and neutral infundin (B. W. & Co.) increase the tonus. Adrenalin will counteract this effect in each case. Neutral "pituitrin" (P. D. & Co.) in a few cases increased the tonus, in others it had no effect, and in many it caused a decrease in the tonus. The decreased tonus is due to the preservative chlorbutanol, 5 mgm. per cc., which is sufficient to cause a loss of tonus. Histamine, and pituitary extracts induce tonus waves even in auricles in which they are not already present.

Terrapin hearts perfused with Ringer's solution pH 7.6, through a cannula placed in the coronary artery, respond to "infundin", 0.1 to 0.2 cc. doses, by increased coronary flow.[5] A similar reponse is obtained upon the injection of 0.1 to 0.2 cc. of N/10 HCl, which is less acid than "infundin." The same heart responds by increased force of contraction, slowed rate of beat, and decreased coronary flow when the pH of the infundin is increased to the same amount as that of the perfusate. A similar response is obtained when pure pituitary extract,[4] dissolved in Ringer's solution and filtered, is injected into the perfusate. Histamine phosphate and argamine acid phosphate (B. W. & Co.), adrenalin (alkaloid), and tyramine acid phosphate cause vasoconstriction of the coronary vessels of the terrapin. Increased tonus, with tonus waves, is observed in the terrapin ventricles in some cases after the injection of pituitary extract and histamine.

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[1] Andrus, E. C., *Am. J. Physiol.*, 1919, xlviii, 221.

[2] Gruber, C. M., and Markel, C., *J. Pharmacol. and Exp. Therap.*, 1918, xii, 45; Snyder, C. D., and Andrus, E. C., *ibid.*, 1919, xiv, 1.

[3] Botazzi, F., *Arch. Ital. Biol.*, 1900, xxxiv, 17; *ibid*, 1901, xxxvi, 277.

[4] Smith, M. I., and McClosky, W. T., *Hyg. Lab. Bull.*, 1924, No. 138.

[5] Sumbal J. J., *Heart*, 1924, xi, 285.

3243

Reduction Potentials of Organic Substances by the Lead Peroxide Electrode.

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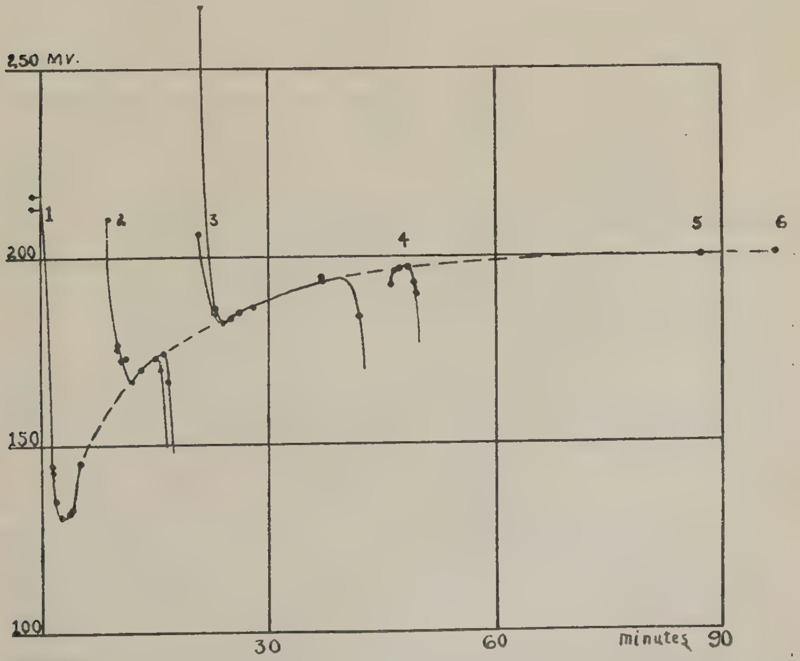
It has been pointed out frequently that the potentials measured in a solution of pure oxidant or pure reductant have a questionable meaning. Moreover such potentials are difficult to measure because they fall in a region of a curve approaching the potential axis asymptotically, and thus a slight change of conditions in the solution causes a wide fluctuation of potential. In such a solution an "inert" electrode, being unpoised, is subject to further fluctuation due to its own surface activity.

The same considerations obviously apply to solutions of those reductants which tend to transform irreversibly into products not active at an electrode, such as most organic compounds. Various schemes have been proposed for the measurement of such potentials, which depend in general upon the addition of some other component which poises the system.

It occurred to us that an oxidizing electrode might in itself have a poising action on an otherwise irreversible system. We have tried manganese dioxide and lead peroxide electrodes. In the following, we report preliminary determinations with lead peroxide electrode on solutions of certain organic reductants. The electrodes were prepared by plating out lead peroxide on platinum electrodes, made anodal in a solution of lead nitrate acidified with eight per cent nitric acid, under voltage sufficient to cause evolution of a fine stream of gas bubbles. These electrodes read in buffer solutions far in the the oxygen overvoltage region. On long standing they approach the oxygen electrode potential, but still read sluggishly in a reducing solution. If placed in a solution of a reducer until equilibrated, then washed in buffer until they again approach the oxygen electrode potential, when next placed in a reducing solution, equilibrium is attained very promptly. The lead is gradually reduced, but as long as the thinnest film remains on the platinum the potentials are constant and precise.

Curves may be plotted by inserting one pair of electrodes after another in the solution, replacing them when a sudden negative change of potential indicates that the platinum surface is exposed. A curve thus consists of a series of short curves, one segment of

FIG. 1.



Curve of potential of 0.1 M dihydroxy acetone at pH 11.3, T = 27° C., as recorded by the lead peroxide electrode. Each full line curve is that of a pair of electrodes, through a segment of which the broken curve can be drawn.

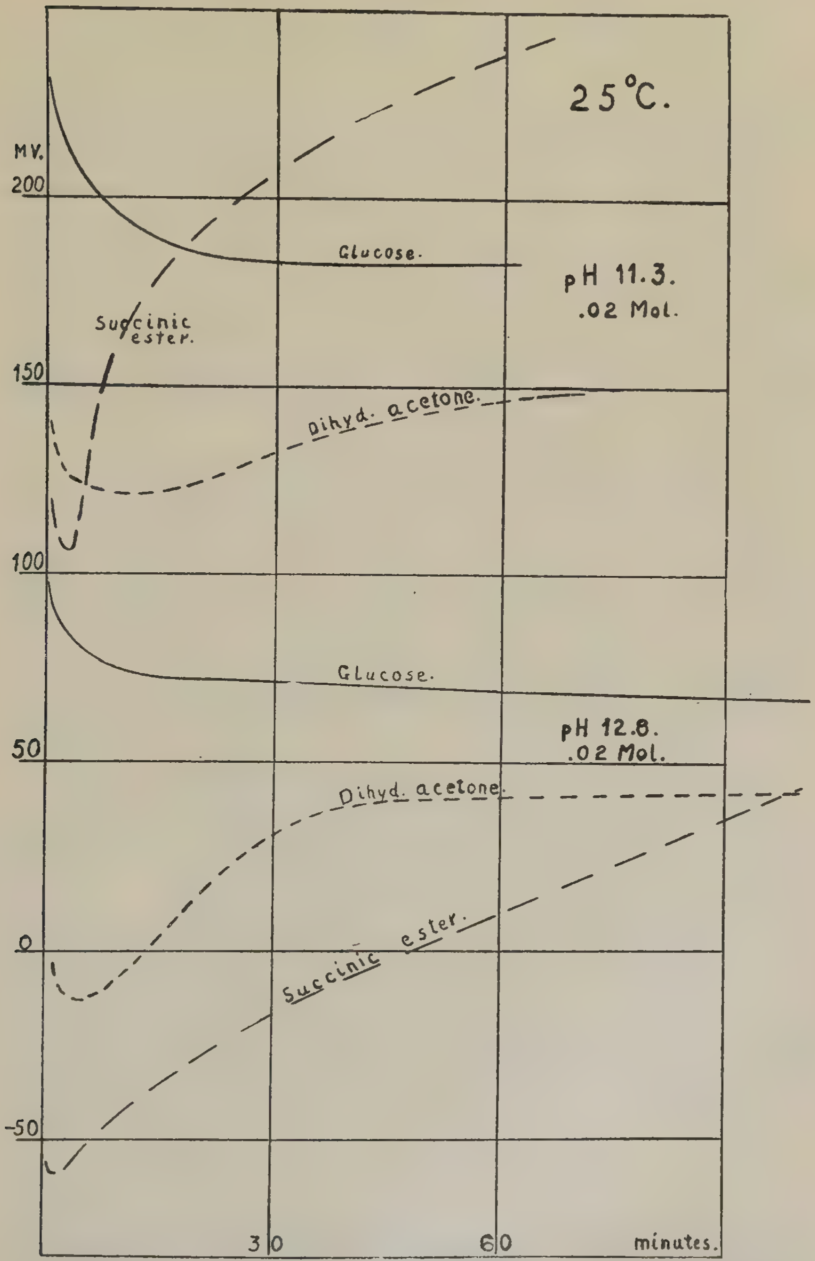
each of which represents equilibrium with the solution. (Fig. 1.) The electrodes are always prepared and used in pairs, and the readings of properly prepared electrodes at equilibrium conditions check to within one or two millivolts. Readings are made in vessels holding fifteen cc. of solution, through which a stream of commercial nitrogen, de-oxygenated by hot copper, stirs the solution and prevents the entrance of oxygen. The rubber stopper to this vessel carries two electrodes. Through a third hole, by which nitrogen is escaping, the capillary end of a tube containing the dry compound to be measured is inserted and nitrogen drawn through it. Then the oxygen free solution is drawn into the tube and allowed to flow back repeatedly until the material is washed into the electrode vessel.

The properties and mode of functioning of these electrodes may be tentatively described as follows:

The reducing substance in solution is a source of electrons which give rise, by reaction with plumbic ions at the electrode surface, to plumbous ions. The solution in the vicinity of the electrode tends to become saturated with both Pb^{++++} and Pb^{++}. When the solution is unstirred this constant concentration of both ions has a poising action on the potential, and enters as a constant into the potential reading.* The reducing substance also tends to give up electrons, through the electrode as a conductor, to the Hg^{+} of the calomel cell, and thus gives a negative potential to the lead peroxide electrode in proportion to the electron pressure of the reducing compound. This reduction potential is thus superimposed upon or added algebraically to that of the poised lead system ($Pb^{++++} + 2e \rightleftarrows Pb^{++}$). The potential measured, therefore, seems to be the resultant of all these forces, namely, the attractions of Hg^{+} and Pb^{++++} for the electrons of Pb^{++} and of the reducing compound. Since the values of Hg^{+}, Pb^{++++} and Pb^{++} are constant, the only variable is the electron pressure of the reducing substance.

In order to test the effect of plumbous ions at the electrode, we saturated the buffer used in certain experiments with $Pb(OH)_2$. The potentials of the unstirred solutions are approximately the same as before. Stirring now has almost no effect on the potentials of irreversible compounds. This indicates that one effect of stirring the solutions to which no $Pb(OH)_2$ has been added is to reduce the concentration of Pb^{++} ions at the electrode surface.

FIG. 2.



Curves of potential of organic reducing substances as recorded by the lead peroxide electrode. Potential as read against a N calomel cell. Further information in text.

Duplicate solutions, in one of which many electrodes are allowed to be reduced, in the other only one, register the same potential at the end. On the other hand, accumulation of reduced ions at the electrode surface should cause a negative potential, which should become more positive on stirring. This is what happens in most solutions, but a very slight stirring is as effective as a vigorous one in raising the potential. The electrodes are generally read under both conditions, stirred and stagnant, two parallel curves resulting. In solutions of reversible substances (ferrocyanide and hydroquinone), stirring lowers the potential instead of raising it, due to the removal of the oxidized form of the substance from the region of the electrode. One other substance which we have investigated (diacetyl succinic ester), also behaves in this way.

In figure 2 are presented curves of three of the compounds we have studied, at two pH levels, one buffer consisting of 0.4 M Na_2CO_3 plus 0.2 M KCl, of measured pH 11.3, the other of 0.1 M NaOH plus 0.9 M KCl, of measured pH 12.8; both are approximately 1.0 molar for the cations. The potentials are in millivolts as read against a 1.0 N calomel cell through a 1 N KCl bridge, the solution contact potentials being neglected for the time being. The electrode vessels are immersed in a water bath at 25° C. up to the levels of the contained solutions. The bath is electrically heated and controlled by an accurate thermostat.

The effect of higher concentration of the reductant is to render the potential more positive in the case of glucose and dihydroxy acetone, and more negative in the case of diacetyl succihic ester. Ferrocyanide and hydroquinone also act like diacetyl succinic ester, as they do also in the effect of stirring. The effect of more alkaline pH is to shorten the time dimensions of the curves of glucose and dihydroxy acetone, as well as to reduce the potential level. The effect on succinic ester is to lengthen the time coordinates of the curve, presumably due to a difference in the course and in the products of the reaction at different degrees of alkalinity.

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* Glasstone (*J. Chem. Soc.*, 1922, cxxi, 1469) found the half-cell $Pt + PbO_2$ (S) + PB (S) + NaOH (N) to be reversible and reproducible. He calculated the values 2.2×10^{-15} for $[Pb^{++}]$ and 1.3×10^{-65} for $[Pb^{++++}]$.

3244

The Fatal Effect of the Complete Loss of Pancreatic Juice.

ROBERT ELMAN and JOHN C. McCAUGHAN. (Introduced by E. Graham.)

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In order to collect the total external secretion of the pancreas, a new method seemed necessary. The older procedures,[1, 2] by which an open fistula was made, permitted the licking of the juice by the animal, and always led to infection of the secretion followed by inflammatory changes in the gland. Such a method was devised in 1923 by Rous and McMaster[3] and was used by them for the collection of the total bile under sterile conditions. We have employed the same technique for the collection of the total pancreatic juice. In brief, a cannula was placed in the large duct of the pancreas in dogs, connected with long rubber tubes leading to the outside and joined to a closed rubber balloon provided with an opening for the periodic collection of the accumulated secretion. The smaller duct or ducts were ligated and cut and omentum interposed between the head of the pancreas and the duodenum to insure against reconstitution.

When mechanical obstruction or accidental infection did not occur the pancreatic juice flowed uninterruptedly and on the fifth to eighth day, quite to our surprise, the animals died with evidences of marked asthenia. The course was similar in all 12 instances studied. For a day or two after operation they appeared normal and ate well. The appetite soon diminished and by the fourth day only water was taken. Vomiting was first noted on the third or fourth day and became gradually worse. They drank large quantities of water which was promptly returned. Asthenia was noted early and increased steadily. The temperature, pulse rate, and respirations remained normal.

The secretion of pancreatic juice continued up to the end, varying, with the size of the animal, between 200 and 500 cc. per 24 hours, In one instance for example (Dog 9, weighing 15 kg.) the amounts collected each morning were 175, 275, 300, 325, and 320 cc. The juice was slightly opalescent, colorless and entirely without odor.

At autopsy, performed in most instances immediately after death, no gross changes were noted. The peritoneum was everywhere smooth and glistening, the outlet tube was firmly bound by omentum, and no infection was anywhere made out. The pancreas seemed a little smaller than normal but was soft and pink. The glass cannula was firmly fixed in the duct. Microscopically the acini of the pancreas were small and surrounded by rather wide clear spaces. The islets appeared normal.

The changes observed in the blood in many instances will be recounted in another communication. Marked dehydration was revealed by hematocrit readings which showed an increase in the proportion of red cells to whole blood as high as 80 per cent, the normal being between 40 and 50 per cent. In addition there was a decrease in blood chlorides and an increase in pH. Blood sugar values were within normal limits and the urine, though repeatedly examined, never contained reducing bodies.

Similar blood findings have been observed after acute pyloric obstruction and death occurs in this condition in about the same time. But the final persistent vomiting in our experiments may alone have been responsible for the alkalosis as, it probably is, in acute pyloric obstruction. But with the loss of so much alkaline fluid it is difficult to understand why alkalosis should occur. Whether the condition bears any relation to that following the establishment of a duodenal fistula where similar findings have been reported must likewise remain unanswered.

It is of interest to note that some of the older observers,[2] even with the open pancreatic fistula, noted the occasional development of hypersecretion followed by death. In our experiments it was the rule. The nature of this insistent and powerful stimulus, which must be one quite apart from the ingestion and digestion of food, may be connected with an unopposed secretion of HCl by the stomach, since acid within the duodenum is a most powerful stimulant of pancreatic flow. This taken with the persistent vomiting may indicate that we are dealing with a condition due to the elimination of the normal regurgitation of alkaline pancreatic juice into the stomach. The partial neutralization of the acid gastric contents is thus prevented. This phenomenon, while long known to occur, has never achieved any great recognition and its existence as a normal phenomenon has even been denied. From these findings and others to follow, it would seem that such a relationship may be of vital significance.

It is a pleasure to acknowledge the helpful criticism and suggestions of Dr. Evarts A. Graham during the course of these experiments.

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[1] Tigerstedt, R., *Handbuch der physiologische Methodik*, 1911, Bd. II, Abt. 2, 174.

[2] Babkin, B. P., *Die äussere Sekretion der Verdauungdrüsen*, 1924, 234.

[3] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1923, xxxvii, 11.

3245

The Bactericidal Influence of Various Substances Upon Gram-Positive and Gram-Negative Bacteria.

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We have found that ether is more rapidly bactericidal in relation to gram-negative organisms *(Escherichia coli, Eberthellia typhi, Eberthellia dysenteriae, Eberthellia paradysenteriae* (Flexner), *Alcaligines fecalis, Salmonella paratyphi, Salmonella Schotmulleri, Pseudomonas aeruginosa, Pseudomonas fluorescens, Proteus vulgaris, Serratia marcescens, Encapsulatus pneumoniae, Neisseria intracellularis)* than in relation to a number of gram-positive organisms *(Staphylococcus albus, Staphylococcus aureus, Staphylococcus tetragenus, Diplococcus pneumoniae* (Types II and III), *Streptococcus pyogenes).* It appears at present that acid fast gram-positive organisms (Mycobacteria), diphtheroids (Corynebacteria) and possibly the Lactobacilli, stand midway between the two first mentioned groups in sensitiveness to this reagent.

We further tested a number of substances containing hydrocarbon chains and possessing varying degrees of lipoid solvent activity on gram-negative and gram-positive bacteria. Our results are shown in Table I.

In general it will be seen that all of these substances act in much the same manner as does ether.

From Table II, it is seen that alkalis act as do the substances containing hydrocarbon chains, but acids act more rapidly on

TABLE I.

Bactericidal Effect of Various Substances upon Gram-positive and Gram-negative Bacteria.

Substances containing hydrocarbon chain.

Bactericidal substance		Gram-positive bacteria	Gram-negative bacteria	Lipoid solvent action
		Killed after	Killed after	
Petroleum ether	C_5H_{12}-C_6H_{14}	>4 hrs.	>4 hrs.	Good
Ligroin	C_6H_{14}-C_8H_{18}	>4 hrs.	>4 hrs.	Fair
Aniline	$C_6H_5NH_2$	5′- 10′	5′- 10′	Fair to good
Phenol, 3 per cent	C_6H_5OH	40′- 60′	20′- 30′	Fair
Cyclohexanol	$C_6H_{11}OH$	10′- 20′	2′- 10′	Good
Ethyl alcohol	C_2H_5OH	40′- 60′	10′- 20′	Poor
Glycerin	$C_3H_5(OH)_3$	>3 hrs.	>3 hrs.	Poor
Chloroform	$CH\ Cl_3$	1′- 3′	1′	Good
Chloroform, 1 part Ligroin, 3 parts		60′- 80′	20′- 30′	
Ethyl ether	$(C_2H_5)_2O$	40′- 50′	5′- 10′	Good
Butyl ether (secondary)	$(C_4H_9)_2O$	90′-120′	30′- 40′	Good
Diphenyl ether	$(C_6H_5)_2O$	120′-140′	100′-120′	Good
Acetone	$(CH_3)_2CO$	<1′	<1′	Good
Acetone, 1 part Ligroin, 5 parts		40′- 60′	20′- 30′	
Ethyl acetate	$CH_3COOC_2H_5$	30′- 40′	5′	Good
Ethyl butyrate	$C_3H_8COOC_2H_5$	<2′	<2′	Good
Ethyl butyrate, 33%		30′- 60′	10′- 20′	

TABLE II.

Bactericidal Effect of Various Substances upon Gram-positive and Gram-negative Bacteria.

Acid, Alkali and Salts.

Bactericidal agent	Gram + Bacteria	Gram — Bacteria
	Killed after	Killed after
$\frac{n}{1}$ HCl	<2′	<2′
$\frac{n}{10}$ HCl	10′- 20′	30′- 90′
$\frac{n}{10}$ Acetic acid	60′- 90′	90′-150′
$\frac{n}{1}$ NaOH	2′- 5′	<2′
$\frac{n}{10}$ NaOH	40′- 50′	2′- 10′
$\frac{n}{10}$ $Ba(OH)_2$	140′-180′	90′-140′
$HgCl_2$ 1:10,000	10′- 20′	10′- 20′
$AgNO_3$ 1:100	10′- 20′	10′- 20′

gram-positive than on gram-negative organisms. Finally we tested two inorganic salts which, however, acted with about the same degree of rapidity on both types of organisms.

Our results so far do not permit of any definite explanation of these differences in the action of the various agents upon the gram positive and gram negative bacteria.

3246

Further Experimental Data on the Sympathetic Innervation and Tonus of Skeletal Muscles.

ALBERT KUNTZ and ALVER H. KERPER.

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In previous communications[1] we reported the results of tonus measurements carried out on the *quadriceps femoris* and *triceps brachii* muscles under normal innervation and following elimination of the sympathetic, in case of the *quadriceps femoris,* and either the sympathetic or the somatic nerve supply, in case of the *triceps brachii* muscles. The results of these measurements indicate that an important component of the tonus of a resting muscle, as manifested by the resistance it offers to passive extension, is mediated through its sympathetic innervation.

The majority of our tonus measurements, the results of which were previously reported, were carried out on cats and dogs under light ether anesthesia. In some instances measurements carried out in this manner were compared with measurements carried out on the same animal while in the waking state. In all these instances the tonus curves obtained while the animal was under light ether anesthesia were essentially similar to those obtained while the animal was in the waking state.

Nevertheless, it has been objected that even light ether anesthesia may vitiate the results of tonus measurements. In order to obviate this objection, and to secure additional checks on our previous experiments, tonus measurements were carried out on a goodly number of animals (cats and dogs) following section of the brain stem just above the midbrain. As observed by Magnus (1916),[2] the tonus of skeletal muscles is not appreciably altered by section of the brain stem at this level. Animals successfully operated in this manner exhibit no voluntary movements, but their spinal and medullary reflex mechanisms remain intact. Spinal and medullary reflexes, especially those involved in progression, are readily elicited, in such animals, as soon as the immediate shock of the operation has subsided.

The tonus curves both of the *quadriceps femoris* and *triceps brachii* muscles with intact innervation obtained following section of the brain stem are essentially similar to the tonus curves of these muscles obtained while the intact animal was under light ether anesthesia. Likewise, the tonus curves of these muscles previously deprived of their sympathetic or (in case of the triceps) somatic innervation obtained following section of the brain stem are essentially similar to the corresponding curves obtained while the animal was under light ether anesthesia before section of the brain stem.

The results of the present series of experiments in general corroborate the results of our experiments in tonus measurement previously reported, and afford additional evidence in support of the theory that an important component of the tonus exhibited by skeletal muscles, in the absence of contractile effort, is mediated through the sympathetic nervous system.

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[1] Kuntz, A., and Kerper, A. H., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 77; 1926, xxiii, 367; *Am. J. Physiol.*, 1926, lxxvi, 121.

[2] Magnus, R., *Pflüger's Arch.*, 1916, clxiii, 405.

3247

The Occurrence of a Physiologically Active Substance in Cultures of the Gas Bacillus.

ARTHUR ISAAC KENDALL AND FRANCIS OTTO SCHMITT.

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Cultures of the gas bacillus which have been enriched with carbohydrate utilizable by the microbe for its energy requirement, contain a substance, possibly substances, which will both induce immediate contracture in isolated guinea pig small intestine immersed in Tyrode solution according to the Trendelenburg technique, and also cause a pronounced, but rather transient, depression of blood pressure in the dog under ether anesthesia upon intravenous injection. The potency of the average 48-hour culture is such that from 0.05 to 0.4 cc. of the fluid medium, freed from bacteria by passage through a Berkefeld filter, will cause such strip of intestine, less than 5 cm. long, preferably from the ileal region, attached to a suitable lever with an arm ratio of 1:9, to record a contracture from 5 to 12 cm. high. Two cubic centimeters of such a filtrate will induce a rather transient drop of some 40 per cent in the blood pressure of a dog weighing about 24 kilos.

The ratio of cultures used to Tyrode solution is from 0.05 to 0.4 cc. of the former to 125 cc. of the latter; and in the dog not greater than 1:1000 of the total blood volume. These effects are elicited by the unaltered, entire, bacteria-free solution which contains much water and other material. The reactive substance is readily washed out from the contracting substance by simply replacing the solution containing it with a fresh Tyrode solution. The actual amount of reactive substance is a very small fraction of the material used for experiment.

The occurrence of the reactive substance is not limited to one or two selected strains of the gas bacillus: some 72 cultures, obtained from the intestinal contents of 41 persons have been studied. Of these, 6 were found to be nearly negative. Quantitative variations were detected among the remaining 66 cultures, but of not great magnitude. The variations in response were, in fact, relatively inconsequential when they were evaluated in terms of the reaction elicited from different pieces of intestines from the same and from different guinea pigs.

None of the bacteria studied thus far, the normal and pathological intestinal group and others, contain demonstrable amounts of this substance. The gas bacillus alone of these contains it, and in relatively large amounts.

The substance which causes the contracture of the guinea pig gut and the transient drop in blood pressure in the dog is produced under quite definite conditions. None appears in the usual cultural mediums from which carbohydrate is excluded, but in media where fermentation is active, the substance is present in considerable—nearly maximal—amount in 48 hours. None is present in the uninoculated mediums.

The substance is not butyric acid, although there is considerable butyric acid produced simultaneously. The butyric acid may be distilled off quantitatively, leaving behind a fluid residuum which contains the substance, apparently increased in potency as the concentration of the mother fluid is increased by the progress of the distillation. Hence, also, it is heat stabile. It should be emphasized that the acid distillate from such a concentration, containing much butyric acid, is wholly inert. The chemical properties and identification of this physiologically active, thermostabile compound are not as yet fully established.

Effects produced by Berkefeld filtrates of gas bacillus cultures upon pieces of small intestine of guinea pig, suspended in Tyrode Solution I 125 cc. I. Time 6 seconds. Gut thoroughly washed with Tyrode between experiments.

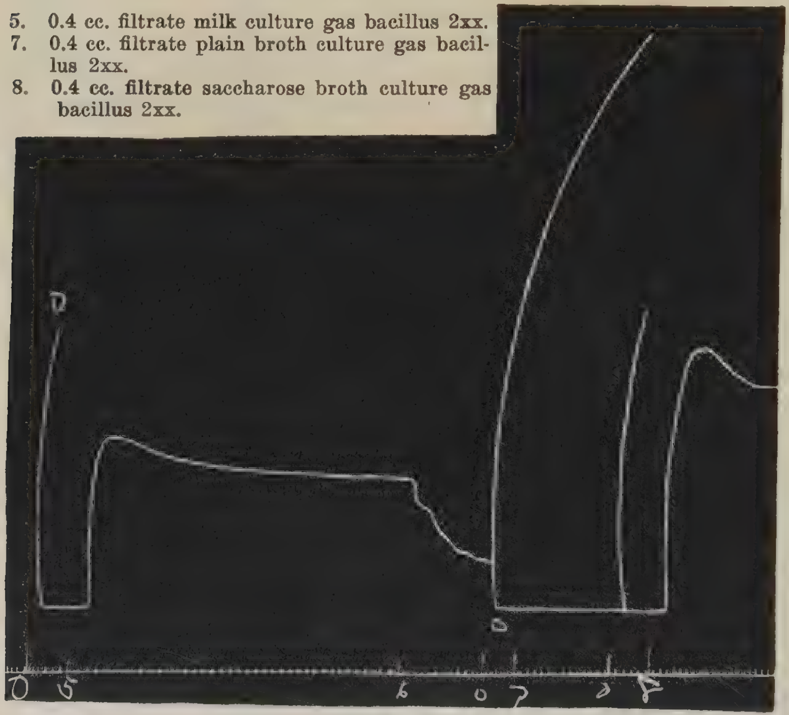
5. 0.4 cc. filtrate milk culture gas bacillus 2xx.
7. 0.4 cc. filtrate plain broth culture gas bacillus 2xx.
8. 0.4 cc. filtrate saccharose broth culture gas bacillus 2xx.

FIG. 1.

At 5, 0.4 cc. filtrate of a 48-hour milk culture added. Note immediate contracture. At 7, 0.4 cc. filtrate of plain broth added. No effect. At 8, 0.4 cc. filtrate of saccharose broth. Immediate contracture. Reactive substance not produced in medium from which utilizable carbohydrate is excluded.

10. 0.4 cc. filtrate milk culture gas bacillus 2xx, neutral to phenolphthalein.
11. 0.4 cc. distillate from 100 cc. of milk culture, gas bacillus 2xx.
12. 1 cc. distillate from 100 cc. of milk culture, gas bacillus 2xx.

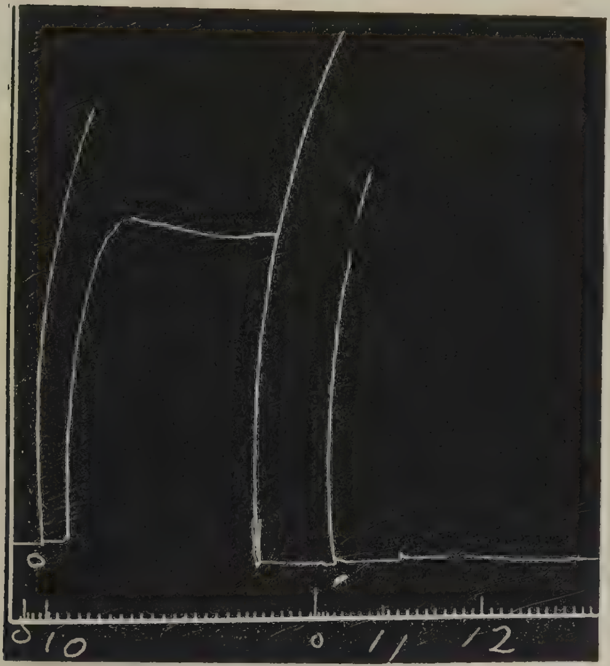

Fig. 2.

At 10, 0.4 cc. filtrate of a 72-hour milk culture of gas bacillus neutral to phenolphthalein is added. Immediate contracture. At 11, 0.4 cc. distillate from 100 cc. of the above milk culture is added. No effect. At 12, 1 cc. of distillate is added. No effect. The reactive substance is not butyric acid and is not volatile.

14. 0.4 cc. boiled filtrate of milk culture, gas bacillus 2xx.
15. 0.1 cc. boiled filtrate of milk culture, gas bacillus 2xx.

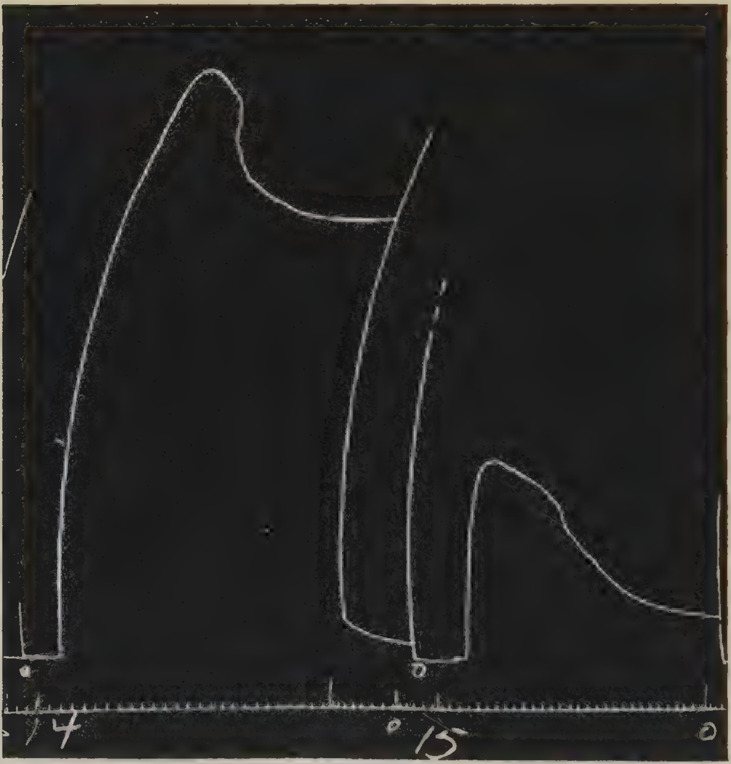

FIG. 3.

At 14, 0.4 cc. of the residual fluid after distillation is added to the Tyrode Solution. I Solution concentrated to 1/3 the original volume. I Immediate and maximal contracture. The reactive substance is thermostable.

3248

Action of the Bacteriophage on a Thermophilic Bacillus.

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Of the various species of bacteria which have been found susceptible to the action of the lytic principle or bacteriophage,[1] it is noteworthy that all are types which have their optimum temperature for growth at 37° C. or lower. It seemed of interest, therefore, to determine whether organisms capable of growth at high temperature—the thermophiles—would also be susceptible to the action of the lytic principle. Efforts to obtain a "thermophilic bacteriophage" have been successful and a preliminary report will be given here.

The organism employed in these experiments was one of the stock collection of thermophiles maintained in this laboratory. It was isolated originally by Tanner and Harding[2] from milk, and has been carried in stock under the designation T60. It is a medium-length, slender, Gram-positve rod, forms spores, and stains readily by the ordinary dyes. The culture is capable of growth at all temperatures from 20° to 60° C., the optimum appearing to be from about 45° to 52° C., at which point growth is very rapid and more luxuriant than at 37°. At room temperature a moderate growth develops after 2 to 3 days.

From sewage polluted river water an active lytic principle was obtained against this organism by employing the usual method of alternate feeding and filtration. The incubation temperature during this process was always 37° C. After developing a lytic filtrate at this temperature it was found to be very active, without any gradual adaptation to a higher temperature, when brought in contact with a homologous culture growing at 52° C. The lytic principle was transmissible through series of broth cultures, one series was held at 37°, the other at 52° C. At the higher temperature lysis occurred more quickly and complete clearing was frequently observed in a few hours.

Lytic areas were produced when appropriate dilutions of the active filtrate were spread over agar slants previously inoculated with the T60 culture. Incubation at 55° C. for 16 to 18 hours resulted in distinct areas 2 to 3 mm. in diameter. The edge of these areas was somewhat irregular, in contrast to the even, round appearance usually shown in the colon-typhoid-dysentery group. Also, the areas showed a central, clear zone of lysis surrounded by a distinct grayish zone of incomplete lysis. Larger areas, 5 or 6 mm. in diameter, were produced by longer incubation. At 37° lytic areas were somewhat smaller than at 55° C. The irregular outline was evident, but the zone of incomplete lysis was absent, or at least so narrow as to escape notice. At room temperature the culture develops slowly and distinct areas are apparent only after 3 or 4 days. Their appearance at this time is quite similar to that shown after overnight incubation at 37° C.

Cross tests in which the T60 lytic principle was allowed to act against three other species of thermophilic bacilli at 52° all proved to be negative. Likewise, similar tests with a Shiga dysentery culture at 37° were negative.

The maximum temperature at which the thermophilic bacteriophage exhibited activity coincided very closely with the limits of growth of the culture. From 45° to 56° C. the culture developed readily, and lysis resulted upon application of a loop of the lytic filtrate. At 58°, although growth was retarded, lysis was evident in those tubes receiving the filtrate. At 60° growth was so scanty as to leave the result of the tests in doubt, while at 62° they were clearly negative.

Tests of inactivation by heat showed that the lytic filtrate resisted temperatures up to and including 70° C., but was destroyed at 75° C. The period of exposure was 30 minutes in each case. It should be noted that in this respect its resistance to heat corresponds very closely to that of the ordinary coli, typhoid, or dysentery bacteriophage. It is an interesting point that the thermophilic lytic principle, which is very active at temperatures fully 20 degrees above the optimum for ordinary bacteria, does not require a correspondingly higher temperature for its destruction.

The present observations may also prove of interest in connection with the discussion of the nature of the bacteriophage. d'Herelle[1] believes it to be a distinct living entity, ultramicroscopic and filterable, and he designates it by a specific name. If the present work is to be reconciled to this viewpoint, we must postulate the existence of thermophilic filterable viruses. Opposed to d'Herelle's concept we have the theories of an autolytic enzyme or some other product of the cell itself. Since the deportment of the T60 lytic principle in regard to temperature follows closely that of the homologous culture, it would seem to lend more support to the latter opinion.

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[2] Tanner, F. W., and Harding, H. G., *Centr. Bakt.*, Abt. II, 1926, lxvii, 330.

ERRATUM.

Volume XXIII, No. 8, "A method for preserving and counterstaining vitally-stained cells," pp. 624 and 625, should read 1.1 (or 1.2) cc. of formalin, instead of 1.8 cc.

SCIENTIFIC PROCEEDINGS.

Massachusetts Branch.

Harvard Medical School, October 13, 1926.

3249

Veratrin Response of Functionally Isolated Muscle Fibers.

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From the Physiological Laboratory, Boston University School of Medicine, Boston, Mass.

Superficial fibers of the frog's sartorius, stimulated with the pore electrode and having their contraction recorded by the mercury droplet method,[1] exhibit a typical veratrin curve when the drug is applied locally in very dilute solutions (1:100,000 or weaker). The quick stroke initiating the response conforms to the all-or-none law governing twitches which prevail in the absence of the veratrin modification. The slow remainder of the curve does not rise above the twitch. It occurs in the same fiber which performs the twitch. Concurrent veratrin and non-veratrin responses may be observed in the same field in different fibers, and may be recorded, on periodic stimulation, as simple twitches superimposed upon a slowly subsiding base-line. Under such conditions of excitation a summation of veratrin responses may occur, resembling the measured, step-like tetanus levels which are brought out by the same method with more frequent stimuli in normal muscle.

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3250

The Behavior of the Arterioles and Capillaries of the Lung.

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In order to study the pulmonary circulation by direct observation and under physiological conditions, a method has been devised which permits a direct view of the vessels of the lung with the chest closed.

Under amytal anesthesia the chest wall of the cat is so prepared that only a layer of pleura remains to keep the chest wall intact. This window of the pleura is planned so that it will lie directly over the extreme edge of the right lower lobe of the lung, in the axillary line. An abdominal incision is made which allows approach to the diaphragm immediately opposite the window in the outer wall. A similar window is then prepared by dissecting the muscle fibers from the diaphram and leaving the pleural layer intact. By means of this window a beam of light is thrown through the lung at sufficient intensity to permit direct observation of the pulmonary vessels with a microscope at the outer window. By employing this method it has been possible to observe the smaller arteries and veins and the capillaries under various conditions.

In every instance the preparation was made under similar conditions, but the appearance of the lung vessels showed marked variation. In some instances 1 or 2 capillaries were open on the wall of an alveolus, while in others 6 or 8 were open and showed circulation in them.

The diameters of the capillaries also varied greatly. At times one saw a capillary of such diameter that only a single red blood cell could pass through, while on the same alveolus or on an adjoining one, a capillary of twice that diameter could be seen.

Pressure upon the abdominal aorta caused new capillaries to open up, and at times arterioles, hitherto unseen, opened. And, as to be expected, when the pressure was removed from the aorta many of the capillaries and in some instances arterioles closed and blocked the circulation through them.

Thus far changes in the O_2 and CO_2 content of the air breathed by the cat, have not influenced the number of capillaries opening and closing. Technical difficulties with the method have also delayed the report of results of the effect of adrenalin, pituitrin, nitrates and other drugs, but these studies are now under way.

3251

Decerebrate Preparation (maternal) for Direct Observation of Unanesthetized Mammalian Embryo.

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From the Department of Neuropathology, Harvard Medical School, Boston, Mass.

In order to study the living mammalian embryo with placental circulation intact and without the disturbing effects of anesthesia, we have made decerebrate preparations of pregnant rabbits under light ether anesthesia. After transecting the thoracic cord to prevent undue reflex movements of hind limbs, anesthesia is discontinued. The uterus exposed by abdominal incision, is opened by longitudinal incision in the relatively avascular region, opposite to the placental attachment. Embryo in transparent amniotic sac is extruded by the contracting uterus, but placental attachment is usually not disturbed. Amniotic sac may now be opened. The embryo is kept moist and warm by a constant drip of warm Ringer's solution. Great care must be used not to place the umbilical cord under tension. A camel's hair brush is useful for manipulation. Such preparations kept moist and warm may be continuously observed for at least two hours and probably longer. Even after the placenta was completely separated from the uterus, some of our small rabbit embryos (9-25 mm. crown-rump length) have survived more than an hour. We believe such preparations will be useful for a variety of studies.

The smaller embryos, up to 13 mm. crown-rump length, are almost completely transparent except for liver and heart, and are particularly suited for transillumination (the heart beat, easily visible to the naked eye, was 50 to 60 per minute in three preparations). For this purpose the embryo with placental circulation intact is placed directly upon the substage condenser of a binocular microscope and transilluminated from below. (It was found convenient to mount the microscope, disconnected from the condenser, on an adjustable stand.)

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*John White Brown Scholar, Harvard Medical School.

We have made the following preliminary observations on the cerebral circulation:

The growth of the cerebral vascular bed from the 9 mm. crown-rump length to the 25 mm. crown-rump length is striking. In the smaller embryo the difference in caliber between main arterial trunk and capillary is relatively slight. After few divisions the artery leads into a simple capillary network which again reunites to form a large vein without apparent anastamosis with other vessels.

Much more complicated is the cerebral vascular bed in embryos of 25 mm. crown-rump length. Here the large arteries and veins are broader and the capillaries appear to be narrower than in the smaller embryos, while the complex capillary bed is separated from the main vessels by a larger number of branches of intermediate size.

In the smaller embryos under magnification of 20 diameters we have obtained beautiful stereoscopic views of the entire cerebral circulation. The deeply placed pulsating arterial flow is readily distinguished from the more superficial and steadily streaming venous flow.

With higher magnifications (100 to 335 diameters) the smallest capillaries appear as minute channels in the cerebral substance through which the red blood cells can be seen to pass but one at a time. Even the endothelial nuclei of the capillary wall may be seen in suitable fields.

This method makes it possible to observe the development and physiology of the cerebral circulation in the *unanesthetized* mammalian embryo with *cranium intact*. Further studies will be carried out in this laboratory.

3252

Certain Physico-Chemical Characteristics of Muscle Globulin.

WILLIAM T. SALTER. (Introduced by Edwin J. Cohn.)

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The significance of the protein constituents of muscle for the contractile process has long been recognized. The instability of these bodies, however, has rendered difficult their characterization. Danilewsky[1] described the extraction of muscle protein with sal ammoniac in 1881. The older method of expression, despite rigid precautions, often led to "denaturation" or "clotting of muscle plasma." The properties of muscle globulin, as of other globulins, are altered both by too low salinity and by too high acidity.[2] Howe[3] has employed phosphate buffers as solvents to overcome these difficulties. In the present investigation an ammonium chloride solution, rendered alkaline by excess of ammonia, was used as solvent and yielded solutions of muscle protein which, for several months, retained solubility in neutral salt solutions.

A globulin-like fraction of ox muscle protein was studied, which presumably corresponded to the "myosin" of von Fürth[4] and the "paramyosinogen" of Halliburton.[5] The method of preparation depended upon: (a) its low solubility in water or dilute salt solution; (b) its greater solubility in the presence of higher concentrations of neutral salt; (c) its precipitation from solutions less than half-saturated with respect to ammonium sulfate. Fresh muscle was finely ground and stirred into a two-molal ammonium chloride solution, to which ammonium hydroxide was slowly added to a concentration one-third normal. This ammonia buffered the acid products and thus prevented "denaturation." The dissolved protein, after centrifugation and filtration, was alternately precipitated by ammonium sulfate, dissolved in more dilute salt solution, and precipitated by further dilution. The reaction was always maintained between pH 8.0 and 8.5 by ammonia. Precipitated by ammonium sulfate, the muscle globulin was freed from albumins[6] and hemoglobin; precipitated by dilution from the serum globulins which dissolve in more dilute salt solutions.

Removal of the excess alkali or of the solvent salt renders the protein less soluble. In the absence of salt, flocculation of the protein occurs over a wide range, extending from pH 4.5 to 8.5, and reaches a maximum, as Collip has reported,[7] in the neighborhood of pH 6.5. The wide flocculation zone of this protein thus coincides with that of the vegetable globulin edestin, and with the zone over which such monoamino monocarboxylic acids as glycine, alanine, and leucine have no buffer effect.

Freed from electrolytes, this fraction dissolved in distilled water, at 25° C., less than 0.025 gram per liter. Water saturated with the protein in this manner had a reaction ranging from pH 6.3 to 6.6.

The addition of alkali to a suspension of isoelectric protein in water first renders it extremely viscous, but finally completely dissolves the protein which combines at saturation with 13×10^{-4} mols of NaOH per gram. Like many globulins,[8] this fraction apparently forms insoluble acid compounds, dissolving only in rather acid solutions and combining at saturation with 15×10^{-4} mols of HCl. The maximal acid- and base-combining capacities of different preparations, calculated from electromotive force measurements,[9] appear in Tables I and II and are in good agreement.

TABLE I. Maximum Acid-combining Capacity.

Preparation and Exp. No.	Protein in one liter (p)	Conc. of HCL (a)	pH	p[HCL]* (pH-pγ)	Conc. of free HCL (b)	Conc. of bound HCL (a-b)	HCL bound per gm. protein $\frac{(a-b)}{p}$
	gm.	N			N	N	mols
VIII A5	7.25	0.018	2.19	2.14	0.007	0.011	0.0015
” A6	”	.020	2.09	2.04	.009	.011	.0015
” A9	”	.022	2.01	1.96	.011	.011	.0015
” A7	”	.024	1.92	1.87	.014	.010	.0014
” A3	”	.026	1.86	1.81	.016	.010	.0014
” A11	”	.028	1.78	1.75	.019	.009	.0012
” A10	”	.030	1.77	1.72	.019	.011	.0015
XIV A12	7.6	0.014	2.67	2.63	0.002	0.012	0.0016
” A13	7.5	.016	2.41	2.36	.004	.012	.0016
” A14	6.9	.018	2.16	2.11	.008	.010	.0014
” A15	6.8	.020	2.10	2.04	.009	.011	.0016
” A16	7.4	.023	2.07	2.01	.010	.013	.0018
					Average		0.0015

*The negative logarithm of the concentration of acid, pHCL, is equal to the difference between the negative logarithm of the hydrogen ion activity, pH, and the negative logarithm of the activity coefficient, pγ. The same relations obtain between pNaOH, pOH and pγ.

TABLE II. Maximum Base-combining Capacity.

Preparation and Exp. No.	Protein in one liter (p)	Conc. of NaOH (a)	p[OH]	p[NaOH] (p[OH]-pγ)	Conc. of free NaOH (b)	Conc. of bound NaOH (a-b)	NaOH bound per gm. protein $\frac{(a-b)}{p}$
	gm.	N			N	N	mols
VIII B5	7.25	0.016	2.11	2.02	0.009	0.007	0.0010
” B9	”	.016	2.13	2.11	.008	.008	.0011
” B13	”	.016	2.16	2.13	.008	.008	.0011
” B2	”	.020	2.06	2.01	.010	.010	.0014
” B8	”	.020	2.00	1.95	.011	.009	.0012
” B12	”	.020	2.07	2.02	.010	.010	.0014
” B4	”	.024	1.96	1.88	.013	.011	.0015
” B7	”	.024	1.85	1.80	.016	.008	.0011
” B1	”	.028	1.80	1.75	.015	.013	.0018
XII 4.5	8.4	0.018	2.20	2.15	0.007	0.011	0.0013
” 5.0	”	.020	2.08	2.03	.009	.011	.0013
” 5.5	”	.023	2.03	1.98	.011	.012	.0014
” 6.0	”	.025	1.94	1.88	.013	.012	.0014
					Average		0.0013

Throughout the physiological range the fraction studied is appreciably soluble only in the presence of salt. Although at reactions alkaline to pH 6.0 this globulin dissolves in salt solutions, at more acid reactions the solvent action of neutral salts is greatly diminished. In this respect also, the behavior of this muscle globulin suggests that of edestin.[8]

This muscle globulin fraction appears to be of a different type than hemoglobin or the serum globulins. All of these animal globulins are more soluble in water than the muscle globulin studied. The solvent action of neutral salts on muscle globulin is, however, more pronounced than on hemoglobin.[10] The globulin-like properties of muscle protein therefore acquire additional significance. Whereas the solubility of hemoglobin is increased only six-fold by neutral salts, the solubility of muscle globulin has been increased several hundred-fold in these studies. Inasmuch as the solubilities of the serum globulins are increased to at least this extent, while their solubilities in water are greater, lower concentrations of salt suffice to dissolve larger amounts of serum globulin than of muscle globulin. Such distinctions have a significance not only for separating these proteins, but for understanding the electrically charged condition of their molecules and their physical state.

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[2] Osborne, T. B., *J. Am. Chem. Soc.*, 1902, xxiv, 28.

[3] Howe, P. E., *J. Biol. Chem.*, 1924, lxi, 493.

[4] von Fürth, O., *Ergebn. Physiol.*, 1919, xvii, 382.

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[9] Cohn, E. J., and Berggren, R. E. L., *J. Gen. Physiol.*, 1924, vii, 45.

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Western New York Branch.

Cornell University, Ithaca, N. Y., October 16, 1926.

3253

The Influence of Ultraviolet Light Upon Blood Platelets in Young Rabbits.

FRANCIS D. GUNN.* (Introduced by B. Roman.)

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Sooy and Laurens[1] have demonstrated that sunlight produces an increase in the blood platelets and the erythrocytes of young rats. Sooy and Moise[2] have produced an increase in platelets in humans, in ten cases of idiopathic *purpura haemorrhagica,* by use of the mercury vapor quartz lamp. The latter authors also cite unpublished experiments of Sooy and Laurens, showing an increase in platelets of albino rats under lamp treatment.

In view of this, an attempt has been made to utilize ultraviolet rays in a tissue study of blood platelet regeneration in rabbits, the full results of which will be reported in the future. The present report deals with platelet and erythrocyte findings in the blood stream.

Methods. In this series 6 rabbits, all under 8 months of age, were exposed daily to the mercury vapor lamp. Treatment began with an initial exposure of 5 minutes at a distance of 16 inches, increased daily by 2 or 3 minutes to a maximum of 30 minutes. The longest period of treatment was 12 days. The backs of the animals were shaved before the first radiation and this was repeated as often as necessary.

Although the direct method of platelet counting has been satisfactorily employed by the writer in older rabbits, the difficulty in taking blood from the small ear veins of these young animals and their somewhat higher normal platelet count, resulted in frequent clotting or clumping of the platelets. Therefore, an indirect or ratio method had to be employed, which is briefly as follows:

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*Fellow in Medicine, National Research Council.

After the rabbit's ear has been shaved, washed and vaselined, a vein is punctured through a drop of the diluting fluid, and as the blood wells into the drop, the mixture is drawn up into a leucocyte pipette, which had been previously filled to about one-third with the same fluid. The pipette is then completely filled with fluid, and the mixture, which consists of blood diluted 3 or 4 times, is shaken 2 minutes. If the flow of blood is very free, less of the mixture and more diluting fluid is used. The fluid consists of 5 cc. commercial formalin, 3 gm. sodium citrate, and 100 cc. physiological salt solution. Films made of the mixture are dried and stained with Wright's blood stain; or, after methyl alcohol fixation, with Giemsa's stain. At least 1,000 red cells and not less than 20 visual fields are counted. If the cells number not more than 80 nor fewer than 20 to the oil immersion field, and if the platelets are clearly discernible, even when lying beneath red cells, the slide is a useful one. During the period of rapid platelet changes 2 or more counts were made daily, at other times, only 1.

Though other advantages of this method could be mentioned, the clearness with which the very small and slender platelets can be visualized, seems its chief recommendation, especially since during the platelet crises the greatest increase seems to be in the small spindle forms.

RESULTS.

The normal average platelet count in these young animals was found to vary between 700,000 and 1,040,000, some animals varying more than others. The greatest variation in any one animal was 530,000 and the least 20,000.

Following radiation a rise in platelets was observed (see charts 1, 2 and 3), the peak occurring between the third and sixth days, and reaching a point between 1,490,000 and 1,890,000. The greatest rise in platelets was 2.3 times the original normal, the smallest rise 1.44 times that number. (See Fig. 2.)

The red cells in every case showed a progressive decrease during the first few days of radiation, a small part of which may be accounted for by slight hemorrhages. In 4 of the 6 animals the red cells began to rise, either just before or just after the platelets had reached their highest peak, the other 2 were not studied beyond this point. In the first 4 animals the original normal red cell count was recovered in 4 to 6 days after its lowest level, and in 3 of them a level of over a million higher than the original red cell count was attained in less than 2 weeks. This rise it will be seen continued for some time after the last radiation. (See Fig. 1.)

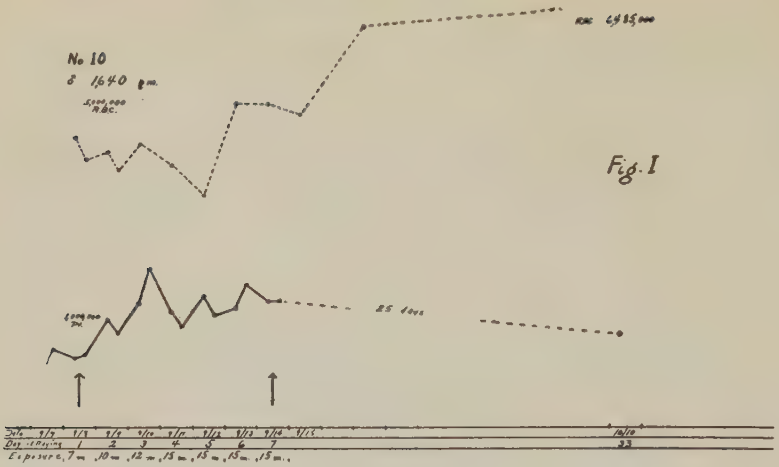

Fig. I

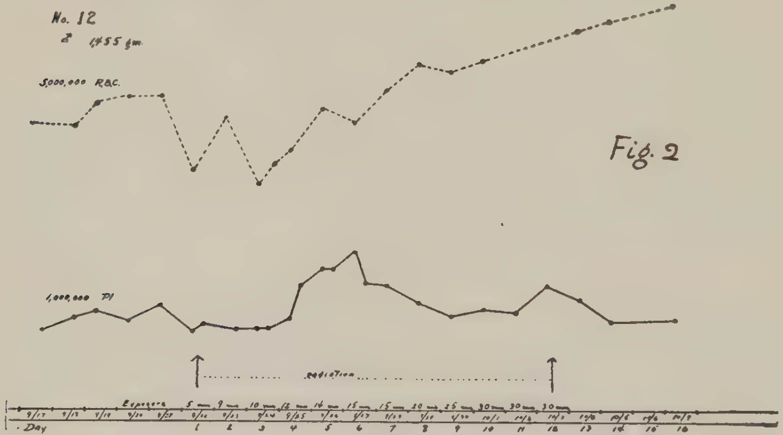

Fig. 2

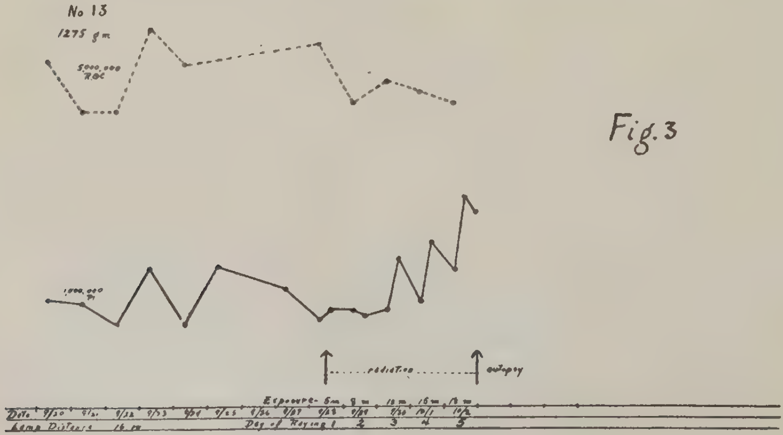

Fig. 3

The leucocytes were apparently unaffected.

SUMMARY.

(1) Exposure to the mercury vapor quartz lamp has produced, within less than a week, a distinct platelet increase, in 6 normal young rabbits.

(2) This increase varied from 40 to 100 per cent of the original normal, but always amounted to at least twice that of the normal variation in the animal. The platelet count returned to normal rather rapidly after having reached its maximum.

(3) The red cells, at first decreased, began to rise at about the time of the platelet peak, returned to normal within 6 days, reached a million above normal in 2 weeks and in 2 cases maintained this level for 3 weeks after cessation of radiation.

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[2] Sooy, J. W., and Moise, T. S., *J. Am. Med. Assn.*, 1926, lxxxvii, 94.

3254

A Seasonal Occurrence of Ketonuria in Fasting Rats Accompanied by Changes in Carbohydrate Metabolism.

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It was found that in the summer months, from the 24th to the 48th hour of fasting, an average of 6.1 mg. of total acetone bodies was excreted per 100 gm. of body weight per 24 hours. The determinations were made by Van Slyke's method. Beginning with late September, the excretion diminished to 1.9 mg. per 100 gm. of body weight per 24 hours. Non-fasting rats excreted 0.4 mg. per 100 gm. per 24 hours. Metabolism experiments, made more or less continuously for a whole year, showed that from October until May the fasting respiratory quotient was in most instances above 0.700, while the opposite was true in the summer months. Since the housing, food and care of the rats was the same throughout the year, the appearance of ketonuria in the summer months was attributed to a seasonal change in fat metabolism. Apparently, in the winter months the fasting rat can oxidize its body fat completely without the simultaneous oxidation of carbohydrates, while this is no longer possible in the summer months. The appearance of ketonuria in fasting rats in the summer months offered a possibility of studying various phases of carbohydrate metabolism during that period.

In the winter months the intravenous glucose tolerance of non-fasting and of fasting rats was found to be close to 2.5 gm. per kilo per hour.[1, 2] In the summer months the tolerance of non-fasting rats remained unchanged, while the tolerance of fasting rats was diminished to 1.6 gm. per kilo per hour or by 36 per cent. In order to decide, whether a decrease in sugar oxidation or in glycogen formation was responsible, recovery experiments were undertaken. In the winter months, from 750 mg. of glucose absorbed from the intestine per 100 gm. of body weight per 4 hours, 281 mg. were oxidized and 388 mg. were converted into glycogen. This corresponds to a recovery of the absorbed sugar of 89 per cent. In the summer months, from 720 mg. of the absorbed glucose, only 72 per cent were accounted for, 140 mg. being oxidized and 380 mg. being converted into glycogen. It is evident that the sugar oxidation in fasting rats is strongly diminished in the summer months. This seems to be the explanation why the intravenous glucose tolerance was found to be lower. Interestingly enough the glycogen formation was not changed. This would be difficult to explain, if one assumes that sugar oxidation and glycogen formation are linked processes. The lower recovery of the absorbed sugar in the summer months may be due to a retention of the sugar as such, or the sugar is retained in some intermediary form on its way to oxidation. This problem will be investigated further. Insulin injections in the summer months led to a better recovery due to an increased sugar oxidation.

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3255

The Absorption of Glycine and d, l-Alanine.

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The rate of intestinal absorption of glycine and alanine has been studied with the method described in a previous publication.[1] Groups of 10 rats were killed 1, 2 and 3 hours after feeding a 15 per cent solution of the amino acid by stomach tube. The average absorption coefficients (*i. e.,* the amount absorbed per 100 gm. of body weight per hr.) for glycine were as follows: 1 hr., 0.048 gm.; 2 hours, 0.050 gm.; 3 hours, 0.046 gm. The values obtained for d, l-alanine were: 1 hour, 0.044 gm.; 2 hours, 0.044 gm.; 3 hours, 0.047 gm. If these values are plotted against time, it will be found that they fall on a straight line. Since the rate of absorption of different hexoses and pentoses was also found to follow a straight line,[1] the same considerations that were made with respect to the sugars, apply to the amino acids. The rate of absorption is, within wide limits, independent of the absolute amount and the concentration of amino acid present in the intestine. If this were not the case, the rate of absorption would be represented by a curve rather than a straight line.

A mixture of equal parts of glycine and alanine was absorbed at a rate of 0.061 gm. per 100 gm. of body weight per hour. One would expect a rate of absorption of 0.09 gm., if each amino acid was absorbed independently from the mixture. It has been found previously,[2] that when glucose and galactose are absorbed from a mixture, the rate of absorption of both sugars is reduced to such an extent that the total amount of sugar absorbed is not much greater than if glucose alone or galactose alone were being absorbed. The same applies to a mixture of glycine and alanine. This phenomenon, which has been called mutual inhibition of absorption, was also present when a mixture of sugar and amino acid was fed. Thus, from a mixture of glucose and glycine in molecular proportion only 0.102 gm. of glucose and 0.041 gm. of glycine were absorbed per 100 gm. of body weight per hour. However, when glucose or glycine are fed alone, the absorption coefficients are 0.180 and 0.048 gm. respectively.

It has been shown recently by several investigators[3, 4, 5] that mixtures of amino acids and sugars undergo marked changes in optical rotation. This has been ascribed to the formation of a sugar-amino acid compound. It seemed of interest to study whether such a combination of sugar and amino acid played a rôle in the absorption of these two substances from a mixture. If this were the case, for each molecule of sugar one molecule of amino acid should be absorbed, irrespective of the molecular proportion in which sugar and amino acid are present in the mixture. However, the experiments did not bear this out. For molecular proportions of mixtures of glucose and glycine of 1:1, 1:0.86, 1:0.6 and 1:0.5, the molecular proportion of absorption was 1:0.96, 1:0.89, 1:0.57 and 1:0.52 respectively. In other words, the molecular proportion of absorption is parallel to the molecular proportion of the mixture. These findings make it very likely that sugars and amino acids are absorbed at the same place of the cell structure.

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3256

Fiber Connections and Functions of the Corpus Striatum in the Cat.

LAWRENCE O. MORGAN. (Introduced by V. E. Emmel.)

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A destructive lesion was placed in the left lenticular nucleus of forty cats. These animals were allowed to live from 9 to 11 days and their symptoms carefully observed. The 15 brains containing the best lesions were then prepared by the Marchi method for degenerated nerve fibers.

The following efferent fiber systems were found to have their origin in the *globus pallidus:*

1. A lateral strio-bulbar fasciculus, closely associated with the lateral cortico-bulbar tract, is composed of (a) a primary, homolateral division to the masticator, facial and ambiguous nuclei; (b) a secondary division to the trochlear, abducens, and hypoglossal nuclei of both sides; (c) a crossed division decussating with the *brachium conjunctivum* to reach the masticator, facial and ambiguous nuclei of the opposite side.

2. A strio-tegmental fasciculus terminates in the interstitial nucleus of Cajal, the nucleus of Darkschewitsch, and the oculomotor nucleus of the homolateral and perhaps also of the opposite side.

3. A large fasciculus of fibers terminates in the intrapeduncular nucleus of Malone (motor division of the *substantia nigra*), and the peripeduncular nucleus of Jacobsohn.

4. A group of fibers passes to the anterior, and another to the posterior part of the *substantia reticularis hypothalami* of Malone (nucleus of Forel's field) of the homolateral side.

5. Many fibers terminate in the subthalamic nucleus of Luys.

6. Many fibers pass to cells in the region medial to Forel's field H_2 (Mammilo-infundibular nucleus of Malone), of the same and opposite sides.

7. Fibers pass through Meynerts commissure to the *globus pallidus* and a few to the region of Forel's field H_2 of the opposite side.

These fiber systems are identical with those found in a study of seven human brains with degeneration in the lenticular nuclei.

A lesion involving the medial division and the ventral part of the lateral division of the *globus pallidus* of the left side was followed by:

1. Left circus movements accompanied by an extreme hypertonicity of the muscles of the left side of the body.

2. A general hypertonic, restless condition affecting all muscles of the body.

3. Constriction of the left pupil.

4. Athetosis in some cases and occasionally tremor.

These symptoms disappear within eight to ten days (except the pupillary constriction) and the muscles that were previously hypertonic become somewhat hypotonic.

A lesion in the dorsal part of the putamen and lateral division of the *globus pallidus* is followed by voice disturbances, and marked difficulties in taking, chewing and swallowing food. These difficulties are at first (8 to 10 days) due to a spasticity of the muscles, but later to a hypotonic condition of the same muscles.

The early symptoms are believed to be irritation symptoms, while the later hypotonic condition is considered to be due to a true absence of function of parts of the *corpus striatum*.

The *corpus striatum* in fish, reptiles, birds and mammals is fundamentally a feeding mechanism. It is concerned with taking, masticating and swallowing food as well as the general body movements essential to searching for and obtaining food, or the pursuit, capture and killing of prey. It may also be concerned with the mating instincts, flight and defense reactions, and some of the more phyletically ancient behavior of the animal as suggested by several investigators.

Pacific Coast Branch.

University of California, Berkeley, October 20, 1926.

3257

Adrenal Hypertrophy in Experimental Uremia.

EATON M. MAC KAY AND LOIS L. MAC KAY. (Introduced by T. Addis.)

From the Medical Department of Stanford University Medical School, San Francisco, Calif.

The adrenal glands, of individuals who die in the uremia of terminal hemorrhagic Bright's Disease, are usually larger than those obtained at post-mortem following death from other causes. An increase in the size of the adrenals, which is probably of the same nature, results from experimental uremia in the albino rat.

The adrenal glands of a group of uremic male rats, (average blood urea 566 mgm. per 100 ml.) 90 days of age, weighed 65 per cent more than the adrenals of their control group (average blood urea 30 mgm. per 100 ml.). This was measured when killed 65 hours after being doubly nephrectomized. Neither group was allowed food during this period. In a group of female rats, 180 days of age, the hypertrophy amounted to 46 per cent during the same length of time. The dried adrenal weights showed less, and the defatted weights a markedly less increase in weight, showing that the increased weight is due in part to lipoid substances.

Preliminary measurements, of serial sections of half of the glands of each experiment, indicate that the volume change is proportional to the weight change, and, that the cortex alone is concerned in the hypertrophy. This cortical hypertrophy, which ensues in experimental uremia, is probably due to the abnormal retention of metabolites, and analogous to the hypertrophy, known to result from chronic infections and following foreign protein injections in the production of antisera. We have recently found a similar adrenal enlargement to take place in the rat, as a result of chronic intoxication with morphine.

3258

Resistance to Morphine in Experimental Uremia.

EATON M. MAC KAY AND LOIS L. MAC KAY. (Introduced by T. Addis.)
From the Medical Department of Stanford University Medical School, San Francisco, Calif.

It is a common clinical observation that uremic individuals often tolerate very large amounts of morphine even in an initial dose. An increased resistance to morphine is also present in experimental uremia.

About 70 hours after being doubly nephrectomized, uremic rats become rigid, shiver, the body temperature drops and they soon die. Sixty hours after the operation they are lively, and, as a rule indistinguishable from their controls. At this time uremic rats show a much greater tolerance for morphine than controls which have undergone identical treatment except for the removal of the kidneys. Subcutaneously the minimum lethal dose for the uremic rats was increased 40 to 100 per cent over their controls. Several of the uremic rats recovered, after 2.0 mgm. morphine sulphate per gram body weight. The increased tolerance was evident whether the morphine was administered subcutaneously, intraperitoneally, intravenously, or by stomach tube.

A relation of the increased morphine tolerance to the adrenal cortex hypertrophy in uremic rats is suggested. Many findings point to a connection between non-specific resistance and the adrenal cortex. In so far as morphine is concerned, we have been able to confirm the observations of Lewis[1] and Scott[2] that tolerance to morphine is decreased following double adrenalectomy. In addition we have found that the adrenal cortex hypertrophies when a rat is given increasing doses of morphine. The hypertrophy is greater the larger the tolerance attained.

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[1] Lewis, J. T., *Am. J. Physiol.*, 1923, lxiv, 506.

[2] Scott, W. J. M., *J. Exp. Med.*, 1923, xxxviii, 543.

3259

Phosphate and Kidney Weight*

LOIS LOCKARD MAC KAY, EATON M. MAC KAY AND T. ADDIS.

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The addition of a certain amount of sodium phosphate to the food of young albino rats is followed by a remarkable increase in the weight of the kidneys, an increase which considerably exceeds that which is found after the ingestion of excessive amounts of other minerals or of protein.

Female rats, 30 days of age at the beginning of the experiment, were given a casein-starch-lard diet in which adequate concentrations of cod liver oil, yeast, wheat germ and salt mixture were incorporated. To other groups of animals the same diet was given, except that part of the corn starch was replaced by acid or alkaline sodium phosphate. The total phosphate (PO_4) concentration in the control diet was 0.68 per cent, in the acid phosphate diet 5.03 per cent, and in the alkaline phosphate diet 5.02 per cent. Measurements of food consumption showed that nearly equal amounts of food were taken by each group. After 44 days the rats were killed. The kidney weights are given in Table I.

TABLE I.

	Number of rats	Average body weight gm.	Average kidney weight mgm.
Control	25	121	898
NaH_2PO_4	24	117	1698
Na_2HPO_4	18	97	1710

No such pronounced increases in kidney weight were seen in similar experiments in which as much or more sodium than was taken in the above experiments was given in the form of sodium chloride or sodium bicarbonate.

Parallel experiments showed that neither acid nor alkaline phosphate had any effect on the degree of compensatory hypertrophy of the kidney after unilateral nephrectomy.

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* This work was aided by a grant from the Ella Sachs Plotz Foundation.

3260

Hastening Development of Female Genital System by Daily Homoplastic Pituitary Transplants.

PHILIP E. SMITH.

From the Department of Anatomy, Stanford University, Stanford University, Calif.

The success of the writer[1, 2] in restoring the atrophied sex apparatus of the hypophysectomized rat by daily homoplastic pituitary transplants, made it seem not unlikely that the transplants might hasten the development of the sex apparatus of the immature unoperated animal. A series of experiments was therefore undertaken in which homoplastic pituitary transplants, taken from full-grown rats of both sexes, were made daily in females which would not normally mature for some time.

It was found that this treatment rapidly induced the changes characteristic of sexual maturity, (opening of the vagina, uterine hyperemia and distension, follicle and corpora formation). These changes were secured as early as the weaning date (22nd day of life), the animals weighing approximately 40 grams. The vaginal smear was oestral in type. Structurally the changes indicative of sexual maturity were present in all parts of the genital system.

When the transplants were commenced at about the weaning date, sexual maturity was induced in 5 or 6 days. If the transplants were begun in animals considerably younger, a longer treatment was required. This was shown by one series (3 treated and 3 controls), in which treatment was instituted on the 14th day of life. One of the treated animals matured sexually in 8 days, two in 10 days. In no case have these transplants failed to bring about sexual maturity at a considerably earlier period than it would normally have occurred. The results obtained transcend all normal variability.

Experiments show that the anterior pituitary component of the transplant is responsible for the induction of the premature sexual maturity.

Kymographic records of the spontaneous contractions of the isolated uterus in Ringer's solution show that the amplitude of these uterine contractions in the treated animals greatly exceeds those of the controls; they, in fact, are equal or nearly equal to those of an animal which matures at the usual age.

In the absence of the ovaries no precocity in the development of the uterus or vagina is brought about by the pituitary transplants, nor is the atrophy of these organs which follows ovariectomy prevented by the transplants. The precocious maturing of the uterus and vagina in the treated unoperated animal is effected by the ovarian (follicular) growth induced by the pituitary transplants.

The precocious maturing of the genital system, which is invariably induced by daily homoplastic pituitary transplants, stands in marked contrast to the failure to hasten sexual development by other methods of pituitary administration. Single transplants of one or several glands have been unsuccessful (Shafer,[3] Clairmont and Ehrlich,[4] Exner.[5]) The intraperitoneal injection of a suspension of the anterior hypophysis of ox, postpones sexual maturity (Evans and Long[6]). A number of investigators have shown that pituitary feeding does not hasten the development of this system.

I have also found that daily pituitary transplants in the adult female usually leads to an increase in the size of some of the follicles of the ovaries. They frequently become cystic. These ovaries may exceed the weight of like glands in the control by from two to four times. The cysts may attain a very considerable size (2 mm.). A thin layer of granulosa cells is present in some of the cysts, in others there is only a thin layer of lutein cells or at places only connective tissue.

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[1] Smith, P. E., *Anat. Rec.*, 1926, xxii, 221.

[2] Smith, P. E., *J. Am. Med. Assn.*, 1926, (in press).

[3] Schafer, E. A., *Berliner Universitätscher.* Teil 3, 1911.

[4] Clairmont and Ehrlich, *Arch. f. klin. Chirug.*, 1909, lxxxix, 596.

[5] Exner, A., *Deutsch. Z. Chir.*, 1910, cvii, 172.

[6] Evans and Long, *Anat. Rec.*, 1921, xxi, 62.

3261

The Effect of Liver Extract on Ducks.

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From the Spreckels Physiological Laboratory, University of California, Berkeley, Calif.

In previous communications[1] it was pointed out that liver extract contained both histamine and cholin, and that the depressor effect was possibly due to these substances. As a further link in the evidence leading to these conclusions the effect of liver extract has been tried on ducks. Hogben[2] has stated that a 1 per cent extract of liver is without effect on ducks, that ducks are less susceptible to depressor bases, and that a pressor effect is more likely, as in rabbits.

The ducks used were fairly uniform in size, weighing about 2 kilos each. Intravenous injections of liver extract (kindly furnished by the Connaught Laboratory) were made in doses ranging from 0.25 to 1 cc. The blood pressure was taken from the carotid artery by the usual method. Such injections are practically without effect on the blood pressure. Sometimes there is a slight rise, sometimes a slight fall (usually due to manipulations), but never the abrupt fall so characteristic of the depressor effect in mammals. As liver extract contains histamine and cholin, the conclusion is still further justified that it is these substances that are responsible for its depressor effect.

Incidentally, it may be stated that Miss Ruth Holzapfel finds radiation with ultraviolet light has no injurious effect on the depressor substance of liver. We know from Ellis and Newton[3] that ultraviolet rays destroy the potency of insulin. It was thought the same might be true for liver extract, if the depressor substance was in the nature of an autocoid. This is a tentative statement as her experiments are yet too few to be conclusive.

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[1] MacDonald, W. J., and Burnett, T. C., *Bost. Med. and Surg. J.*, 1926, cxciv, 381; Burnett, T. C., *Am. J. Physiol.*, 1926, lxxviii, 449.

[2] Hogben, L. T., *Quart. J. Exp. Physiol.*, 1926, xv, 155.

[3] Ellis, M. M., and Newton, E. B., *Am. J. Physiol.*, 1925, lxxiii, 530.

3262

White Blood Cells of the Normal Guinea Pig.

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From the George Williams Hooper Foundation for Medical Research, University of California, Berkeley, Calif.

Investigators have long since realized the importance of the guinea pig as an experimental animal. A criterion of normality in the blood picture of this animal is a necessity to establish a standard for comparative research. Many studies of the differential count of guinea pig blood have been attempted, but, the variations have been so great that a generalization concerning the average count has been impossible. The cause of these divergences may have been due to the breed of the animal, to the physical condition of the animal, to the morphological criteria used for classification, or to the fact that the experimental series were too small. It is our belief, for reasons to be brought out later, that the animals in certain cases were by no means normal, in other cases the morphological criteria were at fault, and in a particular thesis conclusions were drawn from an experimental series consisting of two animals.

The experiments under investigation suggest that the variability, in percentage cell counts, in a series of over one hundred counts, on 40 guinea pigs, is not as great as the differences in the total cell counts given by various investigators. The greatest differences in these observations occur in lymphocyte and neutrophile distributions, as is shown in the following tabulation of results:

	Lymphocytes per cent	Neutrophiles per cent	Eosinophiles per cent
1. Betancès[1]	30-35	40-50	1
2. S. H. Burnett[2]	47.3	31.5	10.7
3. Kanthack and Hardy[3]	24	62	2-3
4. Kurloff[4]	30-35	40-50	10
5. V. Schilling[5]	65-70	25-30	1-3

We have calculated statistically the normal distribution of cell types. Our data indicate that the lymphocyte count is between 70 to 80 per cent, the neutrophile between 12 to 18 per cent, and the eosinophile count between 1 to 2 per cent. We have also calculated the occurrence of Kurloff bodies in the mononuclears and find them to occur in approximately 1.5 per cent of the total cell count, while that type of cell which contains them averages between 5 to 7 per cent of the total cell count. It has been found that great variations from the central tendency of the statistical distribution may be considered as an indication of infection, since in acute infections the neutrophiles are increased depending upon the severity of the infection.

The investigator working with guinea pig's blood is also confronted with the problem concerning the nature and function of a large granular inclusion, known as the Kurloff body, which is found in certain of the mononuclear leucocytes. Haematologists have had great difficulty in explaining this structure. It, however, seems possible that the present available methods of study can be used successfully to explain its origin and formation.

Some of the more important theories which have been advanced concerning the formation and structure of the Kurloff body follow: Kurloff[6] explained its presence by considering it a vacuole filled with substance secreted by the cell; S. H. Burnett[7] considered the vacuoled stage in the formation of the Kurloff body to be a degenerating mononuclear leucocyte; Weidenreich[8] said that it was formed by aggregations of coalesced vacuoles; Ciaccio[9] believed that the Kurloff body was caused by nuclear extrusions; Patella, Foa, and Carbona[10] at one time considered it to be a symbiotic protozoan; Patella[11] later supported the view that the structure was superimposed on the cell and not contained within it.

The experiments indicate that the Kurloff bodies are the nuclei of phagocytized cells and not the accumulation of extra-nuclear material from the cell itself, since we have been able to follow the evolution of this structure through all of its stages both in supravitally and in fixed stained preparations. A detailed discussion of these investigations will be forthcoming at an early date.

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[1] Betancès, L. M., *La Granulation Azurophile*, 1918.

[2] Burnett, S. H., Clinical Pathology of the Blood of Domesticated Animals, 1908.

[3] *Ibid.*

[4] *Ibid.*

[5] Schilling, V., *Folia Haematologica*, 1909, vii, 225.

[6] Weidenreich, F., Leucocyten, 1911.

[7] Burnett, S. H., Clinical Pathology of the Blood of Domesticated Animals, 1908.

[8] Weidenreich, F., Leucocyten, 1911.

[9] *Ibid.*

[10] *Ibid.*

[11] *Ibid.*

3263

Effects of Caffeine and of Paraldehyde Upon the Color of the Frog.

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Incidental to studies on caffeine tolerance in the frog, it was noticed that frogs placed in 1:3000 caffeine solution for 30 to 90 minutes became darker than the controls in water. To test the matter further, frogs were made pale by placing them in water, in glass jars, on a white background, before the window or a strong electric light, as described by Hogben.[1] Caffeine was then added to one jar to make a concentration of 1:3000, a second jar containing water was used for the control frogs. The frogs in caffeine always became dark while the controls remained pale. The results were the same with frogs *(R. pipiens)* in spring, summer and fall of the year. Brown frogs of this species responded to caffeine with a more marked color change than did the green frogs. Injection, of one-half cubic centimeter of 0.7 per cent NaCl solution containing 0.08 g. caffeine, into the abdominal lymph sac of 5 frogs of about 20 g. body weight also resulted in darkening. Hogben[2] reported that caffeine injections had no effect upon the color of frogs.

Microscopic examination revealed the fact that the webb melanophores were contracted before placing the frogs in caffeine solution, but were expanded after the action of caffeine was in evidence.

Several investigators have shown a close relationship between the pituitary gland and the pigmentary system of frogs.[1] Therefore the entire pituitary gland was removed as carefully as possible by the method of Hogben,[3] except that a hand drill was used to bore through the skull. The fact that the operated frogs became pale and remained pale when placed in darkness and moisture, was taken as evidence that the gland had been successfully removed. Such hypophysectomized frogs, when placed in caffeine solution, were definitely darkened, but to a much less degree than the unoperated frogs. The webb melanophores were expanded to a slight degree, short processes, seldom branching, being sent out by the cells. Pieces of webb excised from hypophysectomized frogs, when placed in 1 :1000 caffeine, however, showed as marked an expansion of the melanophores as did the webb of a normal frog, the processes of the cells forming a dense reticulum.

Because of the difficulty of removing the base of the skull without injuring the hypophysis, no such operative controls were attempted.

Although it seems established that the melanophores of the frog have no nervous mechanism, nerve degeneration experiments were carried out. The cutaneous nerves to one side of the skin, in the trunk region of several frogs, were cut. Five weeks were allowed for degeneration. When these frogs were placed in caffeine, the skin of both sides darkened with equal intensity, indicating the absence of a nerve mechanism in the color response of frogs to caffeine.

Normal frogs, placed in 1 per cent paraldehyde solution, also showed marked darkening, while hypophysectomized frogs remained pale in the solution. Webb melanophores of the unoperated frogs were markedly expanded while those of the operated frogs remained strongly contracted. Degeneration of the cutaneous nerves to one side of the trunk did not prevent the darkening due to paraldehyde.

Incomplete microscopic studies of the skin of the dorsum of the trunk of two hypophysectomized and one normal frog indicate that perhaps other factors than contraction and expansion of melanophores play a part in the color changes described above. The intact dorsal skin was observed under the low power of the microscope. In the pale state of the frog, it was observed that the epidermal melanophores were contracted, and that what were assumed to be dermal melanophores were also contracted. The condition of the xantholeucophores could not be determined by this method. After darkening of the skin of the unoperated frog by caffeine or paraldehyde, or of the skin of the hypophysectomized frogs by caffeine, the epidermal melanophores had sent out processes, but no other microscopic changes in the skin could be made out. It is possible that the darkening of the skin was due to the contraction of the xantholeucophores, allowing the more deeply seated black pigment to show up, as suggested by Smith.[4] The changes in the epidermal melanophores would probably not affect the gross appearance of the skin, since, these melanophores are very small and scattered. The changes in the epidermal melanophores are of interest in view of the fact that Hooker[5] reported that they do not contract.

From the above results it is apparent that the pituitary gland influences the response of the skin both to caffeine and to paraldehyde. Further studies are necessary before the site and manner of the influence can be definitely established.

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[1] Hogben, L. T., The Melanophore Effector System, 1924, Oliver and Boyd, Edinburgh and London.

[2] Hogben, L. T., *Biochem. J.*, 1922, xvi, 5.

[3] Hogben, L. T., *Quart. J. Exp. Physiol.*, 1923, xiii, 177-9.

[4] Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1918-19, xvi, 78.

[5] Hooker, *Science*, 1914, xxxix, 473.

3264

Changes in the Reaction of Resting Saliva Produced by Different Micro-Organisms.*

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Dental literature contains many references to the deleterious effects of acids in the saliva, especially after ingestion of excessive amounts of carbohydrate. Those who believe that caries is a process which begins primarily on the external part of the tooth structure and extends inward, stress the solvent action of acids in saliva upon tooth enamel, (largely inorganic) and, subsequently upon the inorganic constituents of the dentine.

Since the reaction of saliva obtained immediately after expectoration as well as that obtained by cannulation is only slightly acid, pH6.6,[1, 2] the combined secretions and excretions of the salivary glands possess too low a concentration of hydrogen-ions to have even the slightest solvent action upon the tooth structure. However, to the actual secretions and excretions of the salivary glands we must add "mouth sweepings," comprising the desquamated cells, food particles, dead bacteria, etc., together with a large variety of living micro-organisms. The importance of the type of food residues, etc., in relation to the predominant micro-organisms in the oral cavity, and, the reaction of the saliva has been emphasized by Bunting.[3]

During the last three years we have been studying the inorganic constituents of the saliva. It seemed desirable to carry out experiments to determine the extreme variations in the reaction that could be brought about by various organisms and with various types of food residues.

METHODS.

Samples of Resting Saliva collected from 3 to 4 adults were combined, and, after removing a small portion for the controls, the main portion was sterilized in Erlenmeyer flasks by autoclaving at 20 pounds pressure for 15 minutes. Except for *B. acidophilus,* the organisms used were obtained by aspiration from the gingival tis-

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TABLE I.

Changes in the Reaction of Incubated Saliva by Various Micro-organisms.

Micro-organisms used	pH OBSERVATIONS					
	Saliva Sample No. 1					
	May 4	May 5				
	8 p. m.	8 a. m.	11 a. m.	2 p. m.	5 p. m.	8 p. m.
Mixed oral (control)	7.1	6.8	6.9	7.0	6.9	6.9
Strep. salivarius	7.1	7.9	8.0	7.8	7.8	8.0
Strep. mitis	7.1	7.7	7.5	7.3	7.3	7.6
Staph. albus	7.1	8.0	8.0	8.0	8.0	8.0
	Saliva Sample No. 2					
	May 6	May 7				
	9 a. m.	9 a. m.	1 p. m.	5 p. m.	9 p. m.	
Mixed oral (control)	7.0	7.1	7.1	7.1	7.2	
B. acidophilus (milk culture)	6.0	5.0	5.0	5.1	5.0	
	Saliva Sample No. 3					
	May 18	May 19				May 20
	8 a. m.	8 a. m.	11 a. m.	3 p. m.	8 p. m.	8 a. m.
Mixed oral (control)	7.1	6.8	6.8	7.2	7.3	7.5
Strep. mitis	7.1	6.8	7.2	7.3	7.4	7.6
Strep. salivarius	7.1	6.7	7.0	7.2	7.3	7.3
Staph. albus	7.1	7.5	7.5	7.8	7.8	8.0
	Saliva Sample No. 4					
	May 19	May 20				May 21
	9 a. m.	9 a. m.	1 p. m.	5 p. m.	9 p. m.	9 a. m.
Mixed oral (control)	7.3	5.0	5.0	5.0	5.0	5.0
B. acidophilus (milk culture)	6.6	5.0	5.0	5.0	5.0	5.0
	Saliva Sample No. 5					
	May 20	May 21				May 22
	9 p. m.	9 a. m.	1 p. m.	5 p. m.	9 p. m.	9 a. m.
Mixed oral (control)	7.3	6.0	6.2	6.4	6.6	6.8
Strep. mitis	7.3	6.1	6.5	6.6	6.6	6.6
Strep. salivarius	7.3	6.7	6.8	7.0	7.5	7.8
Staph. albus	7.3	8.0	8.0	8.0	8.0	
	Saliva Sample No. 6					
	May 24	May 25				May 26
	9 a. m.	9 a. m.	1 p. m.	5 p. m.	9 p. m.	9 a. m.
Mixed oral (control)	6.8	5.0	5.0	5.0	5.0	5.0
B. acidophilus	6.4	5.0	5.0	5.0	5.0	5.0
	Saliva Sample No. 7					
	May 24	May 25				May 26
	9 p. m.	9 a. m.	1 p. m.	5 p. m.	9 p. m.	9 a. m.
Mixed oral (control)	7.6	5.0	5.0	5.0	5.0	5.0
Strep. mitis	7.6	5.8	sample lost			
Strep. salivarius	7.6	7.6	6.8	7.2	7.2	—
Staph. albus	7.6	8.0	8.0	8.0	8.0	8.0

PROTOCOLS.

Saliva sample No. 1; collected May 4, 1925, from two women and one man.

Saliva sample No. 2; collected May 6, from same subjects as above.

Saliva sample No. 3; collected May 18, from three women.

Saliva sample No. 4; collected May 19, from two women and one man. Each subject chewed taffy prior to collecting the saliva.

Saliva sample No. 5; collected May 20, from three women. Taffy candy eaten prior to collecting the saliva.

Saliva sample No. 6; collected May 24, from two women and one man. Each subject ate one soda cracker just prior to collecting saliva.

Saliva sample No. 7; collected May 24, from two women and one man. Each subject ate one soda cracker just prior to collecting the saliva.

sues of human subjects. (We are indebted to Dr. T. D. Beckwith of the Department of Bacteriology for these cultures, as well as for many helpful suggestions.) After inoculation, the samples and the controls were incubated at 37.5° C. Small samples were removed aseptically at various times. The pH determinations were made colormetrically, using appropriate phthalate or phosphate buffer solutions with one of the sulphonephthalein indicators. These buffer solutions were carefully prepared from recrystallized salts and stored in paraffined pyrex flasks. (We are indebted to Mr. K. L. Carter for the preparation of these buffers and also subsequent checking against a hydrogen electrode.) The results of these experiments are presented in the following table:

From the above data it can be seen that only in the controls (mixed organisms of unsterilized saliva) and in the *B. acidophilus* cultures did the changes in the hydrogen-ion concentration become at all significant, coming to a standstill at pH of 5.0. Since we had to resort to mass inoculation (from milk culture), with acidophilus, we cannot definitely attribute the change to the action of the organisms upon the constituents of the saliva. The amount of milk introduced would markedly change the type of the media. In saliva samples 4, 5, 6, and 7, where an excess of carbohydrate was present. it can be seen that the mixed organisms of the mouth are also capable of reaching a pH of 5.0. These changes appear within twelve hours incubation.

It does not seem reasonable to assume that the saliva (excreted and secreted) can ever reach a hydrogen-ion concentration sufficient to have an appreciable solvent action upon the inorganic constituents of the tooth. However, through the action of micro-organisms upon the food particles carried in the saliva, there may be inaccessible places (between the teeth, carious areas, etc.) where the hydrogen-ion concentration would be sufficient to cause solution of the tooth. Direct determinations of the reaction of the saliva, and, *in vitro* experiments such as we have reported would not give any information as to the reaction in such localized areas.

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[1] Starr, H. E., *J. Biol. Chem.*, 1922, liv, 43, 55.

[2] Bunzell, H. H. Bulletin No. 1, Colgate & Co., 1923.

[3] Bunting, R. W., *J. Am. Dent. Assn.*, 1924, xi, 64.

3265

Occurrence of Schick Negative Reactions in Absence of Free Antitoxin in Blood Stream.

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The object of this communication is to present some observations concerning the relationship of antitoxic immunity to the Schick test.

The Schick test, consisting of the intra-dermal injection of 1/50 M. L. D. of diphtheria toxin, shows under proper conditions, a reaction at the site of injection in the non-immune (positive), and a complete absence of reaction in those who are immune (negative). The generally accepted explanation of a negative Schick test is that antitoxin is present in the blood serum, the circulating antitoxin reaching and neutralizing the toxin before tissue damage results.

This explanation is of course plausible, especially since we know of no product of the tissues capable of neutralizing toxin, except antitoxin. There was no ground for dispute of this hypothesis, so long as the presence of antitoxin in the blood was predicated, but it now develops as a result of a series of comparative observations with the Schick and the Kellogg[1] tests that there are individuals who give absolutely conflicting results, in that a negative Schick is accompanied by a lack of antitoxin in the blood as shown by the Kellogg test. These persons have all, with one exception, been found in the group of those originally Schick positive and who have become Schick negative by the administration of toxin-antitoxin. This may have a bearing on the explanation of the phenomenon, and points the way toward a line of investigation.

Attention was first directed to this matter, when some children in the California School for the Deaf and Blind, who had been immunized and later found to be Schick negative, were inadvertently included in a group that was having blood samples taken for the Kellogg test. These were positive, a result that was so surprising that a retest of 14 children who had been immunized five months before, was made by both methods. Two were found to be Schick positive, one was doubtful and the rest negative. All were Kellogg positive.

The tests that furnish the basis of the present report were made at the Sonoma State Home for the feeble minded, through the courtesy of Dr. F. O. Butler, the medical superintendent.

As a result of a fairly large series of comparative tests, it was noted that more than 20 per cent of those who were Schick negative following toxin antitoxin immunization were Kellogg positive, that is, they either had no antitoxin in the blood, or the amount present was too small to account for a negative Schick reaction. Some of these persons have been followed up with subsequent tests for confirmation and to observe whether the condition was temporary or permanent.

The following records are given as representative for the group studied:

Case J. F.—Schick positive,	April, 1923.
Toxin-antitoxin given (3 doses),	August, 1923.
Schick test positive,	March, 1924.
Second series toxin-antitoxin given,	April, 1924.
Schick test negative,	March, 1925.
Schick negative and Kellogg positive	May, 1925.
Again Schick negative and Kellogg positive,	March, 1926.
Again Kellogg positive,	May, 1, 1926.
Case R. M.—Schick positive,	April, 1923.
Toxin-antitoxin given (3 doses),	August, 1923.
Schick test negative,	March, 1924.
Schick negative and Kellogg positive,	May, 1925.
Schick negative and Kellogg positive,	March, 1926.
Again Kellogg positive,	May 1, 1926.

The Kellogg test, while adjusted to detect the presence or absence of 1/30 unit of antitoxin per cc., shows the presence of antitoxin in amounts as small as 1/100 unit, without readjustment of the toxin dosage. It may be, however, that even smaller amounts may possibly, in exceptional individuals, still give a negative Schick reaction, although the explanation of such behavior is not clear. Perhaps there is some difference in tissue permeability that permits larger amounts of lower grade antitoxin to come into contact with the test dose. If this were true, one would expect to find the same thing in persons originally Schick negative, thus giving rise to conflicting results in the primary application of these tests. No such conflict has been observed in a large series of comparative tests.

The question of the exact immune status of these individuals presents itself, with the problem of the explanation of the mechanism involved in the suppression of the necrosing action of toxin in the absence of demonstrable antitoxin. This will involve the almost impossible task of following a large number of persons under conditions of natural exposure to infection.

Closely related to this problem is the question of the origin of antitoxin, latent immunity, local tissue immunity, etc.

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[1] Kellogg, W. H., *J. Am. Med. Assn.*, 1922, lxxviii, 1782; *Ibid.*, 1923, lxxx, 748.

3266

Physiological Phenomena at the Time of Flowering.

H. L. VAN DE SANDE-BAKHUYZEN. (Introduced by C. L. Alsberg.)

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In order to study the growth curve of wheat, and especially the physiological phenomena at the time of flowering, Hard Federation wheat was grown in sand cultures under constant conditions. The illumination was continuous (Mazda C lamps), the temperature varied 1 to 2° C., the relative humidity of the air varied about 10 per cent, the water and salt content in the sand was kept as constant as possible. Every two days 6 plants were harvested and the fresh-weight and dry-weight of every leaf, stem and ear was determined in every plant separately. Each plant had 5 leaves and 1 stem with ear at maturity (all the tillers were removed as soon as they started to grow). These experiments quite confirmed the results which Kreusler[1] obtained with corn some fifty years ago. He found that the gain in dry-weight was minimum at the time of fertilization of the ears or the first development of the kernels. However, recalculation of his results and of the data of several other investigators shows a close correlation between the time when this minimum occurred and the time when 100 per cent female inflorescences were present, *i. e.,* the week during which the maximum number of female inflorescences flower. In our experiment with wheat the minimum occurred exactly at the time of stamination.

This recalculation shows, furthermore, that coinciding with the minimum gain in dry-weight, there was also a minimum gain in fresh-weight or water (fresh-weight minus dry-weight). This minimum is not due to the fact that less dry-weight was gained during that period because the gain of water per gram dry-weight gained, (gain water)/(gain dry-weight), also showed a marked minimum. The gain in water is, therefore, affected more than the gain in dry-weight, so that the relative water-content of the plant decreases suddenly. Whether there is an absolute loss of water or only a relative one, depends merely on the question whether or not the water held in the new tissue formed balances the loss of water in the plant as a whole, *i. e.,* it depends on the absolute gain in dry-weight during that time. In our experiment there was a considerable absolute loss of water exactly at the time of stamination.

Kreusler explains the minimum of increase in dry-weight by the assumption that the increased respiration at the time of fertilization destroys a large quantity of the organic matter gained by photosynthesis, this being normal during this period, so that the net gain is subnormal. This assumption, however, does not take into account the loss of water in the plant at this time. This loss of water occurs in the leaves, the stems, ears and roots. Now several investigators[2] have found that a decrease in water-content of leaves lessens photosynthesis. Therefore, the loss of water in the leaves must be one of the main causes of the minimum gain in dry-weight. The fact that the roots show a minimum as well, proves that the absence of a growing point is not the only cause of the phenomenon.

Under field conditions certain other phenomena occurring at this period come into play. As a result of the decrease in water-content the lower leaves begin to die off, so that at this time the total leaf-area begins to decrease. The gain in dry-weight per unit leaf-area ($cm.^2$), or, per gram leaf dry-weight, is, however, minimum at the time of flowering as well, proof that the minimum of total gain in dry-weight is not caused by this decrease in leaf-area.

After the time of flowering, which we may call a critical period in the life cycle, the relative water-content of the plant decreases continuously, and the leaves gradually die off from the base towards the top of the plant, so that finally the plant dies. The external meteorological conditions at the critical period must, therefore, largely affect the velocity with which the plant will dry out. We may expect, therefore, a favorable effect of cool and cloudy weather (lessening transpiration) upon the length of the period between flowering and ripening and also upon the final yield. This is exactly what meteorological statisticians[3] have claimed for several plants, such as corn, etc. J. W. Smith found that the correlation coefficient between rain fall and corn yield was maximum 10 days after blossoming (+0.74 ±0.05).

The primary cause of the death of an annual which has flowered, is thus the considerable loss of water at the time of flowering, which results in a decrease of assimilatory capacity of the leaves and their gradual dying.

A study of the literature also shows that in other plants, such as potatoes, white mustard, barley, etc., not only can a minimum of increase in dry-weight at the critical period be observed (as pointed out by Kreusler), but also a loss of water. In sugar beets, however, the relative water content of the leaves remains about constant throughout the first year; in the second year the same phenomena occur as in the first year in annuals.

Briggs, Kidd and West,[4] in analyzing Kreusler's results, claimed that at the time of the earliest stages of flower development a decrease in relative growth rate (followed by an increase) occurred, possibly due to increased respiration during this time. We cannot agree with their results, because:

1. They calculated the relative growth rate in a way which leads to erroneous conclusions, as pointed out by Fisher.[5]
2. They assumed that the critical period happened at the time of the first appearance of the inflorescences. This, however, is long before flowering.
3. The increase of the relative growth rate, which follows the decrease, as found by these authors, can be explained by high temperature at this time.

The relative growth rate is not the expression of a simple physiological phenomenon, but the product of two independent variables: (a) the assimilatory capacity per $cm.^2$ leaf-area (productivity); (b) the quotient of leaf-area and total dry-weight of the plant, which we propose to call structural efficiency. The study of the relative growth rate as a product of these two variables lead to an interpretation of the growth curve, which will be published later.

Hitherto, not enough attention has been paid to the relative water-content of plants at different stages of growth. The only way to study it is to carry out experiments under constant conditions.

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[1] Kreusler, U., *Landw. Jahrb.*, 1877, vi, 759; 1878, vii, 536; 1879, viii, 617.

[2] Dastur, R. H., *Ann. of Bot.*, 1925, xxxix, 769.

[3] Smith, J. W., Agricultural Meteorolgy, Rural Text-Book Series, New York, 1920, p. 164.

[4] Briggs, Kidd and West, *Ann. of Appl. Biol.*, 1921, vii, 103, 202.

[5] Fisher, R. A., *Ann. of Appl. Biol.*, 1921, vii, 367.

Illinois Branch.

University of Illinois Medical College, Chicago, October 27, 1926.

3267

Comparison of Albumins of Chicken Egg and Blood by the Precipitin Reaction.

LUDVIG HEKTOEN AND ARTHUR G. COLE.

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Various fractions of chicken egg albumin were prepared from the whites of fresh eggs by both the acid precipitation method of Hopkins and the evaporation method of Hofmeister. These were then tested with a serum against chicken blood albumin prepared from a sample of blood albumin, which had been carefully separated from the other blood proteins. The results obtained indicate that there is a definite immunological relationship between the albumins of chicken egg and of chicken blood, and furthermore, that the albumin of egg white may be resolved into several fractions which differ widely in their titer against a precipitin serum for chicken blood albumin.

3268

Urinary Proteins: Further Studies on Proteins of Nephritis.

LUDVIG HEKTOEN, WILLIAM A. THOMAS AND WILLIAM H. WELKER.

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Our studies[1] on urinary proteins in nephritis were continued by subjecting these proteins to recrystallization. Proteins from 2 of our 10 cases were carried through the fourth crystallization. The protein from the mother liquor of the fourth crystallization, in both of these cases, showed no precipitin reaction with anti-serums for Bence-Jones protein, blood serum euglobulin and blood serum pseudoglobulins. This failure to react with antieuglobulin and antipseudoglobulin serums indicates that this protein fraction is different from that of the crystals of the second crystallization. These proteins react somewhat differently immunologically from the protein of the main crop of the fourth crystallization in their behavior toward antialbumin serums. Work is being continued on these proteins in an attempt to learn more about their relationships.

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[1] Welker, W. H., Thomas, W. A., and Hektoen, L., *J. Am. Med. Assn.*, 1926, lxxxvi, 1333 and 1334.

3269

Plasma Calcium Raising Principle of Bovine Parathyroid Glands.

WILBUR R. TWEEDY. (Introduced by F. C. Koch.)

From the Hull Laboratory of Physiological Chemistry of the University of Chicago, and the Department of Physiological Chemistry, Loyola University School of Medicine.

The method herein described has been found to extract the active principle from bovine parathyroid glands in highly concentrated form.

Two to three grams of finely divided acetone, dessicated, defatted glands are triturated with one and one half volumes of three per cent hydrochloric acid, transferred to 1x8 Pyrex tubes, placed in a water bath, and maintained at a temperature of 70 to 75° C. for 20 minutes.

The extract is made faintly alkaline to litmus, then 0.3 per cent hydrochloric acid is added until a degree of acidity is produced such that the precipitate assumes a flocculent form, and settles rapidly, leaving a clear supernatant fluid.

Filtrate I is made up to a volume of 100 to 150 cc., adjusted to a pH of 5.5 to 5.8, and, sufficient anhydrous acetone added to bring to a concentration of 60 to 70 per cent in acetone. After standing 12 to 15 hours in the ice chest, precipitate II is separated from filtrate II.

Filtrate II is evaporated to dryness *in vacuo* at 40 to 45° C. The residue is dissolved in two and one half per cent trichloracetic acid, corresponding in volume to filtrate II. A finely divided precipitate slowly develops, and after standing 12 to 15 hours in the ice chest, is separated by centrifugalization, or if still in suspension, by filtration. It is then dissolved in strong alcohol and precipitated with anhydrous ether.

Eight to 20 milligrams of such a preparation have been found to produce an increase of 4 to 5 milligrams, in the plasma calcium of dogs ranging in weight from 12 to 19 kilograms, within 12 to 15 hours following subcutaneous injection.

The active principle has been extracted by liquid ammonia from the residue obtained by the evaporation of Filtrate II. It has also been extracted by ethyl lactate and by ethyl butyrate. It is not adsorbed by kaolin at pH values of from 1 to 5, although at higher values it appears to be adsorbed, but has not been released.

3270

The Histogenesis of the Thymus as shown by Tissue Cultures, Transplantation and Regeneration.

NICKOLAS W. POPOFF. (Introduced by A. A. Maximow.)

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The histogenesis of the thymus is still unsettled. The majority of investigators, on the basis of the work of Hammar and Maximow,[1] consider the thymus to be a lymphoepithelial organ in which the original entodermal epithelium of the primordium transforms into a reticulum and is infiltrated with common lymphocytes. Some workers have recently resuscitated the old idea of Stöhr according to which the small thymus cells merely resemble the lymphocytes in appearance and actually represent peculiarly modified epithelial cells.[2] Besides, the epithelial nature of the reticulum seems to be doubtful, mainly because in vitally stained animals, as some authors claim, the reticular cells store vital dyes in the same way as histiocytes.

The method of tissue cultures was applied by us to the study of the thymus problem. Pieces of the thymus were taken from newborn, half-grown and full-grown rabbits and cultivated in plain and carminized plasma with the addition of bone-marrow or embryonic extract.

In the deeper parts of the explant necrosis occurs; the peripheral layers of the lobules and sometimes patches of the medulla remain alive. During the first 24 hours the cultures show a massive emigration of the small cells which behave exactly like lymphocytes. The interstitial connective tissue also serves as a source of migrating lymphocytes, histiocytes and fibroblasts. During the second and third days, as a result of autolysis of the dead lymphocytes and emigration of the living ones, the volume of the lobules decreases, and a broad, pale epithelial layer develops on the periphery through the condensation of the reticular cells. Islands of epithelium eventually arise also in the debris of the deeper layers through contraction and collection of epithelial cells. All epithelial cells are connected by processes. Between them a varying number of dead and live lymphocytes remain to the latest stages. At the same time everywhere in the epithelium mitoses appear. During these changes in the epithelium the small vessels, which are very indistinct in the normal thymus, become more prominent. The epithelium recedes from them and they are now seen surrounded by broader or thinner layers of connective tissue cells of embryonic character, by singly scattered phagocytic carmine-storing histiocytes or macrophages, and by myelocytes. The true epithelium does not store the carmine. Thus in the cultures of early stages the most important relation between the epithelial reticulum and the vessels, with the accompanying connective tissue elements, becomes clearly manifested. The cells storing vital dyes in the normal thymus do not belong to the epithelial reticulum, but to the connective tissue of the adventitia of the blood vessels. After six, eight days of life *in vitro* proliferating epithelium forms strands and islets, sometimes showing intercellular and intracellular secretory vacuoles. The outlines of the epithelial cells are always sharp and no trace of their transformation into lymphocytes or connective tissue cells could be found. The bodies of Hassall may remain unchanged or they disappear through degeneration and phagocytosis by histiocytes. Whether new ones are formed or not *in vitro* can not be stated decisively.

In the oldest cultures (20 days) the epithelium undergoes progressive atrophy, becomes smaller and the vacuoles disappear. Gradually the connective tissue completely overgrows the epithelium.

The experiments with autotransplantations according to the method used by Jaffe were made on half-grown rabbits; the transplants were excised at 2, 4, 6, 8, and 10 day intervals, fixed in Zenker-Formol, embedded in celloidin, and stained with Hematoxylin-Azur II Eosin. The regeneration of the remains of the thymus was also studied.

During the first six days the transplants show degeneration of lymphocytes in the central area. The epithelium forms, on the periphery of the lobules, a capsule-like layer containing dead and live lymphocytes. The vessels, with the accompanying embryonic connective tissue cells, myelocytes and histiocytes, become more prominent. The surrounding local connective tissue shows the usual signs of aseptic inflammatory reaction, infiltration with special leucocytes, mobilization of histiocytes, emigration of lymphocytes and monocytes. It is impossible to discriminate between this local connective tissue and the interstitial tissue of the transplant. The connective tissue with its enlarged vessels penetrates into the transplanted tissue, resorbs the necrotic masses and breaks up the epithelium into islands of different size and shape.

After 7 days regeneration becomes very distinct. The epithelium presents itself in the form of thick growing lobated buds containing many mitoses. The vessels accompanied by embryonic connective tissue penetrate deep into the folds between the epithelial buds. Wherever the connective tissue comes in contact with the epithelium the cells of the former become mobilized as large, medium sized and small lymphocytes. The latter penetrate by means of ameboid movements between the epithelial cells and show many mitoses as do also the epithelial cells. In many places the lymphocytes give rise to special myelocytes; these are mostly of the darkly nucleated, micromyelocytic variety. After 8 days the lymphocytes outnumber the epithelial elements.

The regenerative processes in the transplants of the thymus duplicate the histogenetic processes of this organ in the embryo. The essential feature in both cases is the infiltration of epithelium with true lymphocytes. The epithelium gradually becomes transformed into a reticulum. At the same time the vessels continue to branch between the epithelial masses and form a network of embryonic connective tissue, penetrating the epithelial network, and serving as a source of lymphocytes and histiocytes.

The regeneration of the stump of the remaining part of the thymus duplicates in all essential features the regenerative process in the transplants.

The lympho-epithelial nature of the thymus and the presence of a perivascular mesenchymal reticulum in this organ are decisively shown by these experiments with tissue cultures, autotransplantations and regeneration. No evidence of the development of the small thymus cells from the epithelium was found.

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[1] Hammar, A., *Arch. mikroscop. Anat.*, 1909, lxxiii, 1.

[2] Maximow, A., *Arch. mikroscop. Anat.*, 1912, lxxx, 39.

[3] Maximow, A., *Arch. mikroscop. Anat.*, 1911-1912, xxxix, 560.

[4] Stöhr, Ph., *Anatom. Hefte*, 1906, xxxi, 409.

[5] Schridde, H., *Cent. alg. Path.*, 1923, xxxiii, N. II, 284.

[6] Dustin, A., *Arch. Biol.*, 1920, xxx, 601.

[7] Winiwarter, H., *Bull. Histol. appliquee.*, 1924, N. I.

[8] Jaffe, H., Gottesman, J., *J. Exp. Med.*, 1926, xliii, 403.

[9] Jaffe, H., *J. Exp. Med.*, 1926, xliv, 523.

3271

An Immune Reaction in Tissue Culture.*

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The method of tissue culture has not been extensively used for immunological purposes. Carrel and Ingelbritsten[1] and Przygode[2] have shown that lymph node, bone marrow and spleen react in tissue culture to the presence of antigens by the formation of specific antibodies.

This report is the first on a series of experiments in which we have cultivated various tissues from normal and immunized animals and have watched the effect of the addition of antigens to the cultures.

In one set of experiments we cultured the lungs of 8 normal rabbits and those of 5 rabbits immunized against pigeon erythrocytes.

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

After allowing the cultures to develop for several days, they were washed in Ringer's solution. Each culture then received a drop of pigeon erythrocytes suspended in bone marrow or embryonic extract. The cultures were carefully observed in the living condition and after fixation at various periods were sectioned and stained. Cultures of rabbit lung are characterized by great numbers of phagocytes; these we consider to be of mesenchymal origin. They are identical with the alveolar phagocytes of the adult lung.

Pigeon erythrocytes when added to cultures of lungs from normal rabbits attach themselves to the phagocytes of the cultures but are rarely taken up by these cells in periods as long as twelve days. The phagocytes in cultures of lungs from immunized rabbits, however, take up great numbers of the erythrocytes in a few hours. No difference was noted in the reactions of the lungs from those rabbits which had previously received one injection, and those which had received two injections of pigeon erythrocytes in the process of immunization.

When inactivated, anti-pigeon-erythrocyte rabbit serum was added to cultures of normal rabbit lung plus erythrocytes in which there had been no phagocytosis, a widespread phagocytosis of the foreign red cells appeared in 25 minutes. This process can be watched under the microscope; it is quite dramatic to see the periphagocytic erythrocytes suddenly pass into the interior of the phagocytes.

Cultures of lungs from normal rabbits reacted quite differently from cultures of lymph node to the presence of pigeon red cells. In the latter the large pigment containnig histiocytes, on reaching the surface of the explant, ingest great numbers of the pigeon erythrocytes. The rate of phagocytosis in the lymph node cultures is, however, much slower than in the lung cultures from immunized rabbits. As a result of our experiments we believe the phagocytosis which takes place in the immunized culture to be due to the presence of an immune body in the serum. In none of our cultures have we found any evidence of the pulmonary capillary endothelium being mobilized into either granulocytes or phagocytes.

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[1] Carrel, A., and Ingebritsten, R., *J. Exp. Med.*, 1912, xv, 287.

[2] Przygode, P., *Wien. klin. Wochsch.*, 1913, xxvi, 841; 1914, xxvii, 201.

3272

Inorganic Blood Phosphate of Rats on Rachitic and Non-rachitic Diets.

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From the Department of Pharmacology and Therapeutics, University of Illinois, College of Medicine.

This investigation of the inorganic blood phosphate was undertaken in order to determine its value as an early diagnostic sign of rickets. The blood phosphate was determined in rats receiving a control diet, the McCollum rachitic diet No. 3143, and the rachitic diet plus various substances which were being tested for antirachitic properties. The fluctuations in the blood phosphate of rachitic and non-rachitic rats due to age, anesthesia and injury were also investigated.

The blood was drawn from anesthetized rats by cardiac puncture, and except in case of the very young rats determinations were made on individual samples. The latest Briggs' modification[1] of the Bell and Doisy phosphate method was employed.

Deep anesthesia increased the inorganic blood phosphate rapidly. Light anesthesia for 7 to 15 minutes increased it only 2 to 6 per cent. The struggling and injury involved in stunning and bleeding the rats, which are methods used by other investigators, caused much greater increases in blood phosphate than any degree of anesthesia necessary to draw blood from the heart.

Values on control diet: The youngest rat upon which phosphate was determined was 6 weeks old. This gave a value of 8.6 mg. per 100 cc. of blood. During the latter part of the growth period the blood phosphate remained above 6 mg. After 3½ months it gradually declined to 4 to 5 mg., showing a reduction of phosphate with age.

Values on rachitic diet: Diet 3143 lowered the blood phosphate of mature rats from 4.2 to 1.8 mg. in 15 to 20 days, and of young rats from 6.25 mg. to 2.3 mg. in a similar period.

Effect on blood phosphate of curative measures:

Irradiation: Irradiation of mature rats on diet 3143 maintained the blood phosphate at a higher level than was the case in the non-irradiated animals, although there was a distinct tendency for this value to decrease. Irradiation of young, rapidly growing rats had no appreciable effect on blood phosphate.

Cod-liver oil: Two per cent cod-liver oil incorporated with the rachitic diet raised the blood phosphate of a mature rat from 1.8 to 3.3 mg. in 4 days, and, 5.2 mg. in 15 days after the addition was made. The blood phosphate of young, growing rats was raised from 2.3 mg. to 4.3 mg. in two weeks and to 5.4 mg. in five weeks. The value for control rats the same age was 6.25 mg. These results agree with those reported by Robison and Soames.[2]

Irradiated cholesterol: The daily administration of 2.5 to 5 mg. of irradiated cholesterol entirely prevented rickets, but had only a slight influence on blood phosphate. Young rats receiving non-irradiated cholesterol showed a similar slight increase in phosphate.

Both irradiated and non-irradiated cholesterol maintained the blood phosphate of mature rats at the normal level, 4.4 to 5.4 mg.

The liquid NH_3 soluble fraction of irradiated cholesterol and of the non-saponifiable portion of cod-liver oil, when fed in amounts of 0.2 and 0.3 mg. daily, entirely prevented rickets, but had no influence on blood phosphate. The Ca values for these same rats were 10.77 and 11.9, and, the products of (Ca)×(P) were 24.7 and 27.37 respectively, both within the rachitic range given by Howland and Kramer.[3]

Control diet after rachitic diet: When animals, which had been on a rachitic diet 2 months, were returned to a control diet, the blood phosphate was doubled in twenty-four hours and reached the high values of 8 to 10 mg. in one week. The increase in blood phosphate after the change from rachitic to control diet seems to be roughly proportional to the severity of the rachitic condition. After a period of 2 to 3 weeks the inorganic blood phosphate of these rats began to decrease, until, at the end of 6 weeks it had reached a normal value of 4 to 5 mg.

The results of this study indicate that while cod-liver oil raises the inorganic blood phosphate almost to normal, direct irradiation of, and feeding irradiated cholesterol to young rats, had very little effect on blood phosphate. It is possible to obtain low phosphate values, and a low product of (P)×(Ca) in animals, which so far as roentgenograms can demonstrate, show a perfect calcification of the bones. The inorganic blood phosphate is influenced by many factors aside from the rachitic condition, many of which we cannot explain at present. We have in mind an investigation of the different forms of blood phosphate and other factors, which may help explain some of these phenomena.

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[1] Briggs, A. P., *J. Biol. Chem.*, 1924, lix, 255.

[2] Robison, R., and Soames, K. M., *Biochem. J.*, 1925, xix, 153.

[3] Howland, J., and Kramer, B., *Am. J. Dis. Child.*, 1921, xxii, 105.

3273

Correlation of Skin and Stomach Tonus.

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In previous papers[1] we have discussed the antagonistic autonomic status of the peripheral region (skin, muscles) as compared to the splanchnic region of the body. These experiments have been made by means of lymph analysis and studies on the leukocytic partition between the two regions. During the course of these studies we have also made observations on the relation of stomach contractions and the coincident changes in leukocytes and body temperature in dogs and in patients.

Chart No. 1.

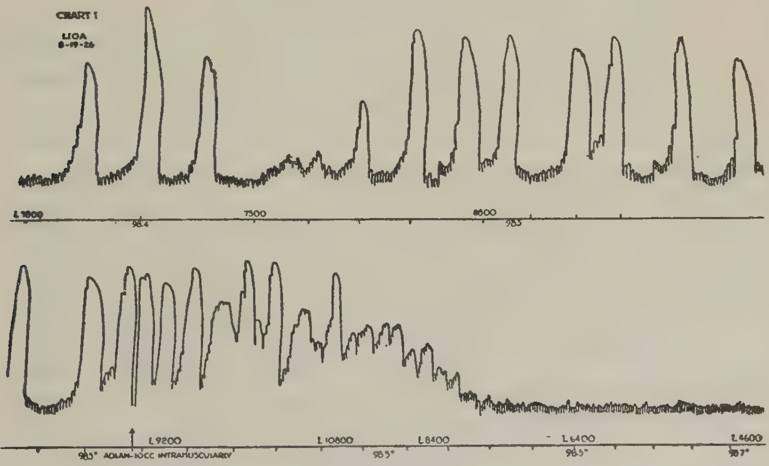

Chart No. 1 illustrates the typical relation of stomach contractions, leukocytes and temperature observed in a patient following the intramuscular injection of aolan.

Conclusions: During the period of hunger contractions the periphery is parasympathetically oriented. Following an intracutaneous or intramuscular injection of aolan, a peripheral sympathetic status is initiated (leukopenia—body temperature increased) and with it a cessation of stomach contractions takes place.

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[1] Müller, E. F., and Petersen, W. F., *Klin. Woch.*, 1926, v, 53.

3274

Varying Hydrogen Ion Concentration upon Rate of Action of Proteolytic Enzymes in Gastric Mucous Membrane.

GRANT M. KLOSTER. (Introduced by F. C. Koch.)

Department of Physiological Chemistry and Pharmacology, University of Chicago, Chicago, Ill.

All experiments were made on neutral water extracts of fresh hog stomach mucosa, prepared by suspending the finely ground mucosa in about a liter of water, and, allowing it to extract for ten to twelve hours in a cool place. The mixture was then filtered through cheese cloth to remove solid material, and the filtrate diluted with water to such a volume that the weight of mucosa in grams should be 5 per cent of the final volume in ccm. The extract was then acidified by the addition of 5 N HCl to pH 3.50, 3.00, 2.50, 2.00, 1.50, 1.00 and .90. At each of these points a small sample was removed for a control, and, a large sample for the experiments. Each of the large samples was made slightly alkaline for 30 minutes, then acidified, first, to a point near its original acidity, when a sample was removed, and finally, to pH 1.00 (approximately the optimum), when a final sample was taken. Mett's tubes containing coagulated egg white or coagulated blood plasma were placed in each of the samples, and the preparations placed in an incubator at 40° C. to digest for 24 hours. The amount of digestion was determined by measuring the column of protein dissolved.

The control series showed, generally, a very slight activity at pH 3.50, which increased slowly up to pH 2.50 or pH 2.00. Above this acidity the rate of digestion increased rapidly, reaching a maximum near pH 1.00, after which there was a definite decrease. In the series which was made alkaline, then acidified to points near the original acidities, there was shown a very slight activity or none at all. The series in which the acidity was brought to pH 1.00, after having been alkaline, showed a progressive loss in activity, the loss increasing with the increase in degree of original acidity. The loss was practically complete toward the end of this series.

Strongly alkaline solutions were found to have a destructive effect upon the unactivated enzyme in the extracts. A study of the effects of concentration of the alkali and the length of time of exposure of the extracts was made, varying the alkalinity from pH 8.06 to pH 12.73, and varying the time from 5 minutes to 1 hour. In the more weakly alkaline solutions a maximum destruction was effected in 15 minutes with no further change in an hour's exposure. The loss was about 5 per cent. In the more alkaline solutions the destruction was nearly complete (96 per cent) in 5 minutes. Much of the loss was due to NaCl formed in the reaction. In fact, about 75 per cent of the decreased action observed in the more alkaline solutions may be ascribed to the NaCl effect.

SUMMARY.

1. The inactive principles of an extract of gastric mucosa can be activated to various degrees by varying the degree of original activity.
2. The activity developed at a given degree of acidity can be destroyed by 30 minutes' exposure to a weakly alkaline solution and cannot be restored on acidification to a point below or equivalent to the original H-ion concentration.
3. Further activity can be demonstrated by acidifying to higher degrees than the primary acidity.
4. The proportionate loss in digestive power is greater, upon making the solution alkaline, the higher the degree of preliminary acidity.
5. The activity shown by an acidified extract of gastric mucosa is dependent in part upon the actual amount of active enzyme present.
6. The effect of alkalinity upon the unactivated extract is to lower the resultant activity after acidification. The effect increases rapidly with increasing alkalinity, and all digestive action may be lost around pH 12 to pH 13.
7. Except for the very slight alkalinity of pH 8.00, this destruction by alkali is rapid, reaching its maximum in a few minutes.

3275

Behavior of Human Gall-Bladder During Fasting and in Response to Food.

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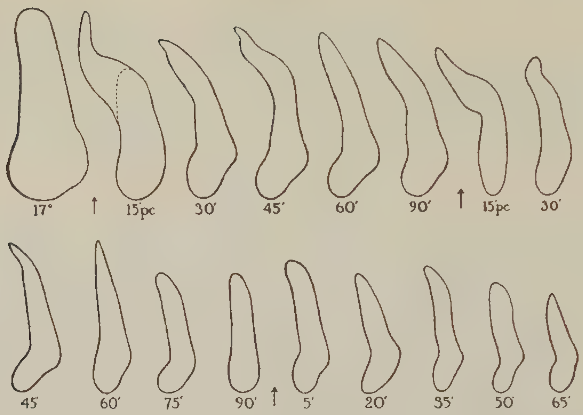
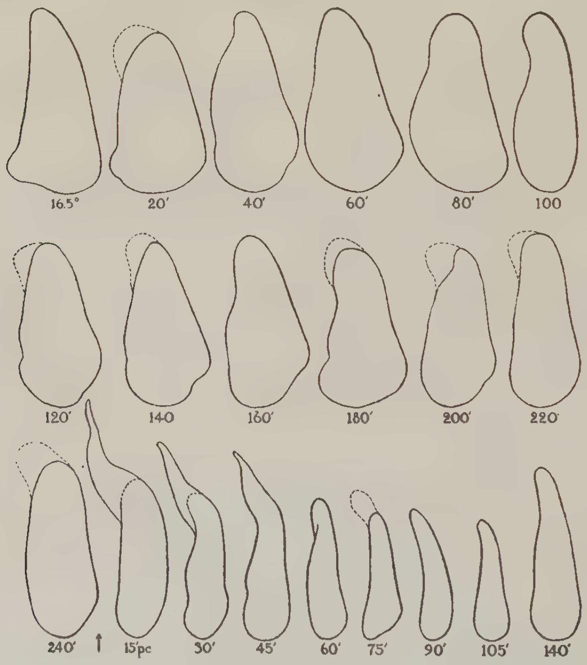
In a recent paper by the author,[1] roentgenological evidence from patients and experimental animals was presented in support of the following conclusions: (1) the mammalian gall bladder, at least in

FIG. 1.



Tracings of human cholecystograms x ¼ (Dr. L. O. Morgan); (three weeks later than those in Fig. 2). 17°-Gall bladder 17 hours after oral administration of iodine salts. Arrows indicate three intervals at which milk was taken.

FIG. 2.



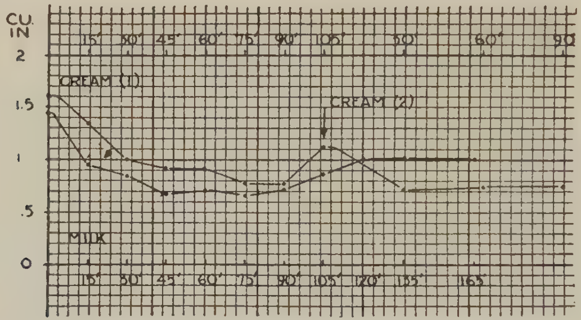
Tracings of human cholecystograms x ¼ (Dr. L. O. Morgan, Sept. 27, 1926). First two rows indicate changing shape of gall bladder during fasting. Dash lines denote ampulla. Note contraction at 100'. Third row shows response to ingestion of half pint of cream. Volume at 240', 2.85 cu. in.; at 60', 0.5 cu. in.; at 105', 0.35 cu. in. of bile.

man and in cats, is able to expel all its fluid contents by the contraction of its musculature; (2) after meals this contraction is so timed as to meet the first requirements of digestion—in contrast to hepatic secretion which is designed to meet later needs; (3) in cats the gall bladder responds to such changes in the circulation as are produced by blood transfusions and to such superficial lesions as are caused by shaving and by cutaneous incisions; (4) adrenalin (with its implication of the sympathetic nervous system) is the most powerful excitant of gall bladder contraction yet found.

One of the methods employed was the making of long series of human cholecystograms at 15-minute intervals after meals. In such series it was found that the first X-rays after food showed changes in shape of the human gall bladder which could be interpreted as due only to muscle contraction. In two new series (with Dr. L. O. Morgan as the subject) this fact has been even more strikingly brought out than before (15′ pc., Figs. 1 and 2). During the first 240 minutes (Fig. 2) the gall bladder assumed the shape of a flaccid organ, subject to distortion by external pressure. Fifteen minutes after ingestion of cream the wall tightened and there was revealed an *actual expulsion of iodized bile into the cystic duct.* During the preceding period of fasting, however, there occurred one marked contraction (100′, Fig. 2), as a result of which the volume of the gall bladder shrank from 4.15 cubic inches to 2.17 (for method of calculation, see former paper). This was the only time when Doctor Morgan complained of hunger pangs, and, the coincidence would seem to corroborate Boldireff's statement[2] that during fasting, bladder bile is discharged into the duodenum synchronously with contraction of the stomach. This finding, together with the writer's discovery that certain changes in the circulating blood cause discharge of bladder bile, raises the question as to whether contraction of the gall bladder may not, at times, subserve another function than that of digestion.

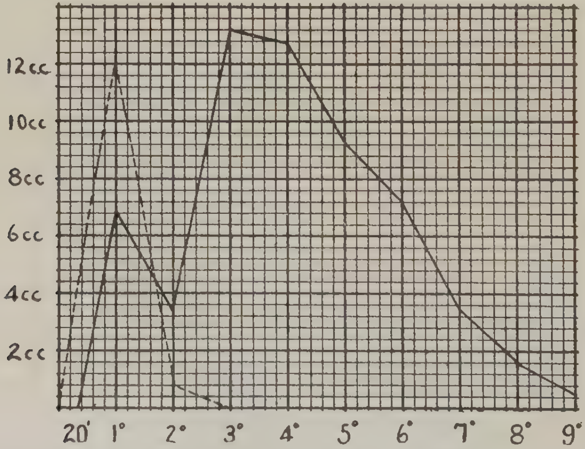
Another problem which has puzzled recent workers in this field, relates to the particular elements in the food which cause excretion of hepatic bile and contraction of the gall bladder. Working with dogs, Klodnizki[3] has shown that egg-yolk causes greater acceleration of bile flow from the liver than any other food, and I have found that in cats the same substance induces the most effective contraction of the gall bladder (indeed being the only natural food which consistently empties that viscus).[4] Although Klodnizki has not analyzed the effect of egg-yolk he has given a curve of secretion of hepatic bile following ingestion of milk (*continuous line,* Fig. 4). In analyzing the reaction to this food he correlates the first flow of hepatic bile with the entrance of neutral fat into the duodenum, and the second (which is by far the greater) with the entrance of split products of milk digestion. In order to test the effect of the latter on the gall bladder, another volunteer patient

FIG. 3.



Graphs from two series of human cholecystograms (R. L. Webb: milk series, lower line, Sept. 23, 1926; cream series, upper line, Oct. 14, 1926). Volumes of bile computed from tracings of gall bladder shadows. Abscissas represent time in minutes after a meal; ordinates, volume of bile in cubic inches. Compare with similar graphs in former paper (foot note 1). ***Arrow,*** **second application of cream.**

FIG. 4.



Graph illustrating rate of biliary flow after ingestion of milk. *Dash line,* amount of bladder bile leaving the gall bladder in milk series of R. L. Webb (computed from lower line in Fig. 3). (In Dr. Morgan's case, Fig. 3, where we were dealing with a large gall bladder, the discharge of bile in the first hour after milk amounted to 37 cc. *Continuous line,* (after Klodinzki) showing amount of liver bile collected per hour, after diet of milk, in bile fistula dogs.

(Mr. R. L. Webb) was given a pint of milk and then X-rayed at frequent intervals for 3 hours (lower line, Fig. 3). In the first hour, over 12 cc. of bile was expelled from the gall bladder; in the second hour, less than 1 cc.; and in the third hour, none (*dash line*, Fig. 4). Therefore, if the rate at which the split products of milk are formed in man at all resembles that for dogs, we must assume that the split products of milk do not induce effective contraction of the gall bladder. The substance which stimulates the biliary reservoir would thus appear to be some element in undigested food, such as neutral fat. But when a half pint of cream was tried on the same individual (upper line, Fig. 3), no greater effect was produced, in spite of the greater amount of fat in cream. Accordingly a comparison of the effect of these two diets suggests that the distended gall bladder is a trigger mechanism which is set off by a moderate amount of neutral fat, the quantity of which is not important beyond a certain minimum. To confirm this idea, periodic feeding of milk was resorted to (Fig. 1). The first pint induced a marked expulsion of bile, lowering the volume in one hour from 2.8 to 0.55 cu. in.; as the gall bladder began to fill, a second pint was given, lowering the volume in the next hour and a half to .33 cu. in.; finally, a third dose diminished the contents of the gall bladder to .17; and it is not unreasonable to assume that further feedings would have emptied the viscus. The effect of these repeated feedings is to reproduce the successive phases of contraction (and eventual collapse of the gall bladder) which the writer has produced by one feeding of egg yolk and cream. From this we may infer that egg yolk is so effective because successive portions of its fatty substance may continue to be discharged into the duodenum for a long period (egg yolk being retained in the stomach of cats for at least 7 hours); and that after the gall bladder has emptied itself (2 to 3 hours after a meal) the subsequent flow of hepatic bile tends to keep the sphincter papillae open and prevent filling of the reservoir. It is interesting to note, however, that ingestion of food is most effective when the gall bladder is distended. Apparently the response of smooth muscle in this organ is greatest when it is stretched (*cf.*, also with the urinary bladder), to which may be added the auxiliary action of abundant elastic tissue in its wall.

This initial reaction to many foods, containing a moderate amount of fat, emphasizes the importance of the gall bladder as an organ of digestion; and since it plays so consistent a rôle, there should be an end to the indiscriminate cholecystectomy of the last decade. The fact that it is not a vital organ is no argument against its utility.

Also, at present, it is equally indefensible to deny its importance on the ground that certain animals normally reach maturity without developing a storage organ for bile. For in such species a different latent period and modified rate of hepatic flow may yet be found to compensate for loss of the *vesica fellea.*

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[1] Boyden, E. A., *Anat. Rec.*, 1926, xxxiii, 201-256.

[2] Boldireff, W. N., *Diss.*, St. Petersburg, 1904.

[3] In Babkin, ''Die äussere Sekretion der Verdauungs-drüsen.'' Berlin, 1914, p. 345.

[4] Boyden, E. A., *Anat. Rec.*, 1923, xxiv, 388; *ibid.*, 1925, xxx, 333.

New York Meeting.

Rockefeller Institute, November 17, 1926.

3276

Observations on the Pathogenesis of Appendicitis.*

M. J. TOBIAS. (Introduced by W. H. Barber.)

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The following experiments were undertaken to study the pathological changes in the caecoappendix which follow interference with its lumen and its blood supply:

TABLE I.
Results Following Ligation of Blood Supply to Caecoappendix.

Exp. No.	Duration of Experiment	Pathological Findings
25	Four days	Areas of infarction in caecoappendix and continuous intestinal loop; microscopically gangrenous inflammation.
31	Ten days	Data incomplete.
51	Fourteen days	Data incomplete.
53	Six days	Adhesions; microscopically, swelling and edema of all coats, hydropic degeneration of muscularis, dilatation of lymph spaces in submucosa and mild hemorrhagic and parenchymatous changes in the mucosa.
56	Four days	As in Exp. No. 53.

Remarks: Excepting experiment No. 25, which shows areas of infarction in caecoappendix and contiguous intestinal loop, there is, grossly, no evidence of serious peritoneal involvement; microscopically this group shows generalized swelling and edema, hydropic degeneration of the muscularis, dilatation of the lymph spaces in the submucosa and mild hemorrhagic and parenchymatous changes in the mucosa. (See Microphotograph of slide No. 56.)

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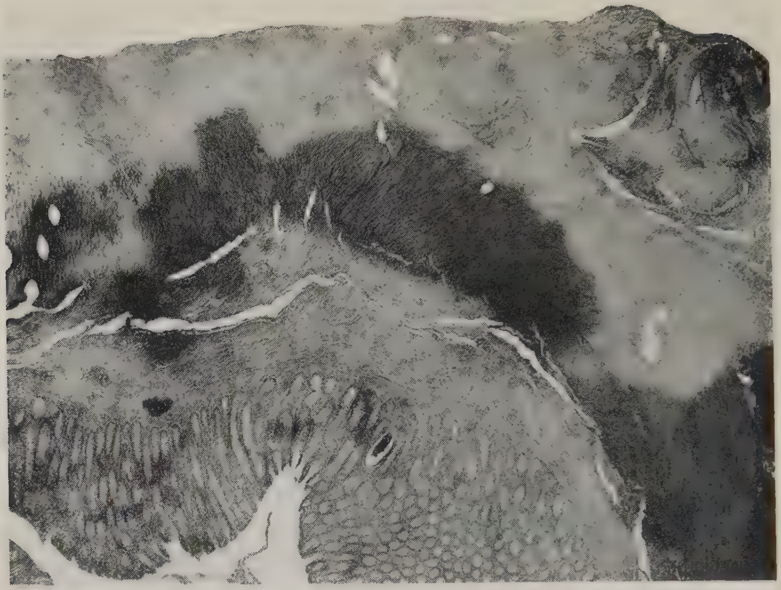
* Submitted as part of requirements in Course of Postgraduate Surgery.



EXPERIMENT No. 56

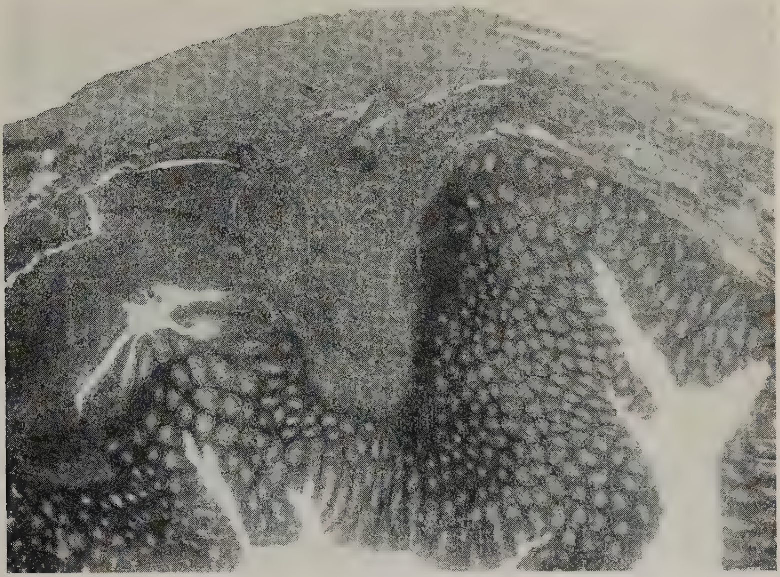
Shows swelling and edema, hydropic degeneration of muscularis, dilatation of lymph spaces in submucosa and hemorrhagic and parenchymatous changes in the mucosa, following ligation of blood supply to caecoappendix.

TABLE II.

Results Following Obstruction of Lumen of Caecoappendix.

Exp. No.	Duration of Experiment	Pathological Findings
10	120 hours	Perforated appendix and generalized peritonitis.
40	168 hours	Data incomplete.
70	48 hours	Adhesions; hemorrhagic inflammation affecting all coats, particularly that of submucous.
76	28 hours	As in Exp. No. 70.
84	13 hours	As in Exp. No. 70.
90	7 hours	As in Exp. No. 70.

Remarks: Experiment No. 10 shows gangrenous appendicitis and generalized peritonitis; in this instance the ligature cut through the appendicial wall, thus simulating the most extreme consequences of rupture of the vermiform appendix in the human. The gross lesions in the others show local adhesive peritonitis, and microscopically hemorrhagic inflammation affecting all the coats of the caecoappendix. This inflammation is particularly severe in the submucosa. (See Microphotograph of slide No. 84.)



Experiment No. 84

Shows hemorrhagic inflammation, particularly marked in submucous coat, following obstruction to lumen of caecoappendix.

TABLE III.

Results Following Obstruction of Lumen of Caecoappendix and Ligation of its Blood Supply.

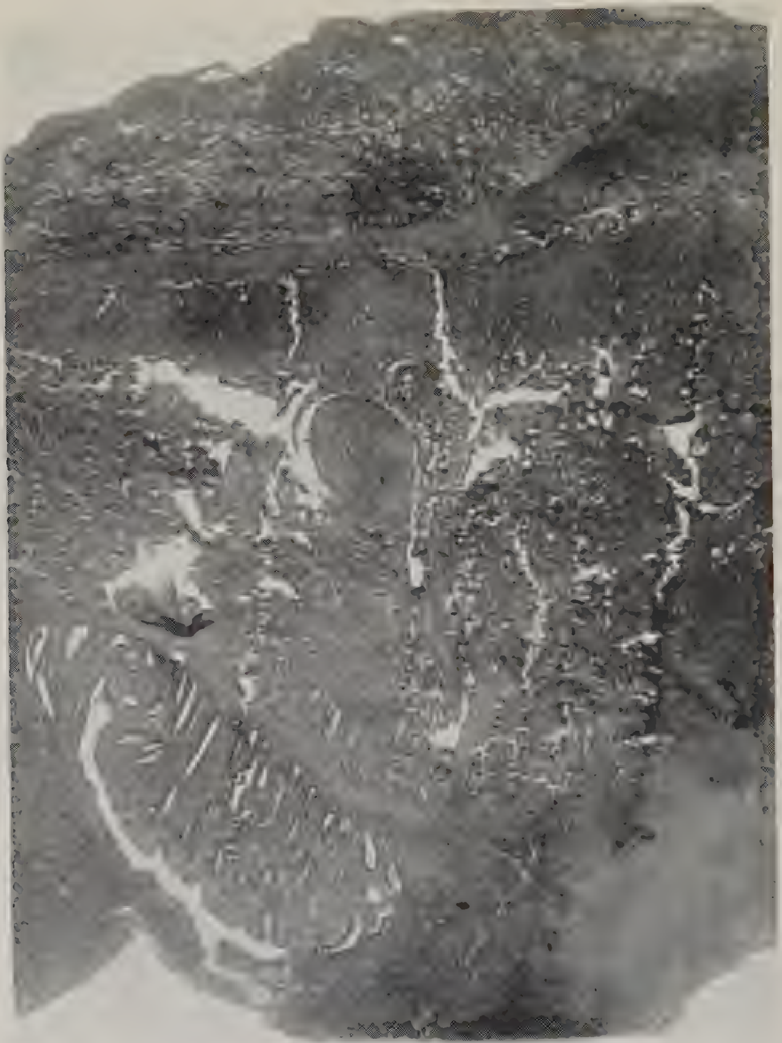
Exp. No.	Duration of Experiment	Pathological Findings
15	Three days	Adhesions; local abscess; phlegmenous and gangrenous appendicitis.
18	Three days	Phlegmenous and gangrenous appendicitis; generalized peritonitis.
20	One day	As in Exp. No. 18.
102	One day	As in Exp. No. 18.
106	One day	As in Exp. No. 18.

Remarks: This group invariably shows gangrenous appendicitis, and, in most instances, generalized peritonitis. (See Microphotograph of slide No. 106.)

Conclusions.

1. Ligation of the blood supply to the caecoappendix in the dog is followed by local adhesive peritonitis; microscopically the organ shows generalized swelling and edema, hydropic degeneration of the muscularis, dilatation of the lymph spaces in the submucosa and mild hemorrhagic and parenchymatous changes in the mucosa.

2. Ligation of the appendicial lumen by linen ligature is followed by localized adhesive peritonitis, and hemorrhagic inflam-



EXPERIMENT No. 106

Shows gangrenous inflammation affecting all coats, following obstruction to lumen and blood supply of caecoappendix.

mation of all the coats of the appendix, but affecting chiefly the submucosa.

3. Ligation of the blood supply to the caecoappendix plus obstruction of its lumen are followed by generalized peritonitis and gangrenous appendicitis.

3277

Gasometric Determination of Blood Calcium.

DONALD D. VAN SLYKE and JULIUS SENDROY, JR.
From the Hospital of the Rockefeller Institute, New York.

The calcium is precipitated and washed in a centrifuge tube as in the Kramer-Tisdall method, and is redissolved in 2 cc. of 1 N sulfuric acid. The solution is transferred to the manometric blood-gas apparatus of Van Slyke and Neill,[1] using sufficient water for washing to bring the volume up to 6 cc. Dissolved air and CO_2 are removed by shaking the solution 2 minutes in the evacuated chamber and ejecting the extracted gases. One cc. of 0.1 N potassium permanganate, previously acidified with 1/20 volume of 0.1 N sulfuric acid, to decompose any carbonate present as contamination, is added. The excess permanganate oxidizes the oxalic acid to CO_2. The chamber is evacuated and shaken 3 minutes. The gas pressure p_1 is read on the manometer with the gas volume at 0.5 cc., if the calcium is being determined in a 1 cc. serum sample, at 2 cc. volume if a 3 cc. sample has been used. After reading p_1 the CO_2 is absorbed with 1 cc. of 5 N sodium hydroxide, and p_2 is read with the same gas volume in the chamber. The pressure due to the CO_2 from the oxalate is p_1-p_2-c, where c is the value of p_1-p_2 determined in a control analysis of the reagents. The CO_2 in millimols is estimated from Table III of Van Slyke and Neill. If a 1 cc. sample of serum is used, the factor in the column for "Sample = 2 cc., $s = 7.0$, $a = 2.0$, $i = 1.014$" is multiplied by $2 \times 1.03/1.014 \times 2.0/0.5 = 0.508$, to obtain the factor giving milliequivalents of Ca per liter; since in the present analysis the sample is ½ as much, i is changed to 1.03, and a is changed from 2.0 to 0.5, only s being the same as on the table. To calculate milligrams of calcium per 100 cc. of serum, the factor in the above mentioned column of Van Slyke and Neill is multiplied by $2 \times 0.508 = 1.016$, since 1 milliequivalent of Ca per liter is 20 mg. per 1000 cc., or 2 mg. per 100 cc. A sample with 0.1 mg. of Ca (the amount in 1 cc. of normal plasma) gives at 20° a pressure reading of 154 millimeters, which can be duplicated within 1 mm. The error is about 1/5 as great as in the permanganate titration of the same amount of calcium.

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[1] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523-73.

3278

Gasometric Determination of Glucose.

DONALD D. VAN SLYKE AND JAMES A. HAWKINS.
From the Hospital of the Rockefeller Institute, New York.

The method depends on the reduction of ferricyanide by the sugar, and the determination of the excess ferricyanide, by measuring the nitrogen gas evolved by the reaction with hydrazine:
$4\ K_3Fe(CN)_6 + N_2H_4 + 4\ NaOH = 4\ K_3NaFe(CN)_6 + 4\ H_2O + N_2$
The reagent solution contains 6 grams of potassium ferricyanide and 150 grams Na_2CO_3 per liter. One volume of this is mixed in a tube of 10 mm. diameter with 2 volumes of sugar solution, or of Folin-Wu blood filtrate, containing 0.04 to 0.4 mg. of glucose per cc. This is heated 4 minutes by immersion in boiling water. The solution is cooled to room temperature, and is resaturated with air by stoppering and shaking the tube 1 minute. Two cc. of a solution, prepared by mixing 1 volume of saturated hydrazine sulfate solution with 1 volume of 40 per cent sodium hydroxide, is placed in the chamber of the Van Slyke-Neill[1] manometric blood gas apparatus. Three cc. of the sugar-cyanide solution are then measured into the chamber from a rubber-tipped pipette (described by Van Slyke and Neill). The reaction of ferricyanide and hydrazine is practically instantaneous. The chamber is evacuated, the mixture is shaken 1 minute, the gas volume is reduced to 0.5 cc., and the pressure in the manometer is read as p_1. A control without glucose is run, and the pressure read as p_0. The decrease in pressure, p_0-p_1, due to reduction of ferricyanide by sugar, is the measure of the glucose. One control serves for an entire series of sugar determinations, which can be run off at the rate of about 1 each 3 minutes. One mg. of sugar causes a drop in the pressure reading of 238 mm. at 15°, 242 at 20°, 246 at 25°, 250 at 30°. The factor per mg. of sugar is constant, within 1 per cent, for amounts of sugar varying within the limits given above for use with the method.

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[1] Van-Slyke, D. D., and Neill, James N., *J. Biol. Chem.*, 1924, lxi, 523-73.

3279

The Respiratory Quotient of Exercising Muscle.

HAROLD E. HIMWICH AND MILTON I. ROSE.

From the Department of Physiology, Yale University Medical School.

Previous work has revealed the fact that the respiratory quotient of amphibian muscle, when excised, is unity both in rest and exercise. The same result has been obtained on excised resting mammalian muscle. On the other hand, in the study of the respiratory quotient of resting mammalian muscle *in situ,* that is, a gastrocnemius muscle with isolated blood supply and intact nervous connections, the respiratory quotient is not unity, but close to that obtained simultaneously of expired air of the entire animal. Therefore, it appeared interesting to see what the respiratory quotient of exercised muscle *in situ* would be.

In our experiments 10 dogs, starved from 5 to 15 days, were studied. The muscles of the lower extremities of these dogs were stimulated by a tetanizing electric current. After 10 and 15 minutes of exercise, and in one case at the end of the fourth minute, simultaneous samples of blood were drawn from the femoral artery and vein and analyzed for CO_2 and O_2 by the method of Van Slyke and Neill. In all cases, the concentration of the blood as revealed by determination of its oxygen capacity was unchanged by passage through the tissues. In most samples of blood there was no change in the CO_2 dissociation curve in arterial and venous blood. In other words, a close approximation to the "steady state" had been established. The average of 16 respiratory quotients was .81 ± .05. Eight determinations, of the respiratory quotient of entire resting animals, by analysis of expired air collected simultaneously with the blood, yielded an average respiratory quotient of .82 ± .04; and in 16 samples, taken from animals while exercising, the average respiratory quotient was found to be .82 ± .07. Since, in the experiments here reported, a small part of the venous return must have come from non-muscular tissues, another series of experiments are now being conducted on gastrocnemii with isolated blood supply.

SUMMARY AND CONCLUSIONS.

The gaseous exchange during exercise was studied in 10 dogs starved for 5 to 15 days. The average of 16 respiratory quotients of the muscles of the lower extremities obtained by analysis of the

blood was .81 ± .05. Eight respiratory quotients obtained from the air during rest yielded an average of .82 ± .04. Sixteen air samples collected from exercising dogs gave an average of .82 ± .07.

The respiratory quotient of the exercising muscle *in situ* is not unity. This indicates that not only carbohydrate foodstuffs but also non-carbohydrate are used in muscular exercise.

3280

Effect of Insulin and of Muscular Exercise on Protein Metabolism.

WILLIAM H. CHAMBERS* AND ADOLPH T. MILHORAT.

From the Department of Physiology, Cornell University Medical College, New York City.

The total urinary nitrogen was determined in hourly periods on normal fasting female dogs weighing 6 to 20 kilos. The urine was collected by catheter and the bladder thoroughly rinsed each time. After 3 preliminary control hours, insulin (usually 5 units per kilo of body weight) was injected subcutaneously and the urine collected for 3 or 4 periods until sugar was given to the animal to relieve the hypoglycemic convulsions.

The changes in protein metabolism after the insulin depended upon the nutritive condition of the animal. After short fasting periods of 1 to 5 days insulin increased the nitrogen excretion. In a typical case, on the second day of fast, the increase was from an average of 99 mg. per hour during the preliminary hours to 137 mg. after insulin. With a longer fasting period of 7 to 14 days, insulin produced no effect, for example 101 mg. per hour before and 100 mg. after the injection. When carbohydrate (50 to 100 gm. of sucrose) was administered every 3 or 4 days to an otherwise fasting animal, the injection of insulin produced the same increase in nitrogen excretion on the 16th as on the 2nd day of fast. Similar results were obtained on a dog in which muscular movement was prevented by amytal anesthesia.

Two fasting dogs were run daily on a treadmill to observe the effect on protein metabolism of decreasing the body carbohydrate through muscular exercise. After 4 hourly preliminary control periods the dogs were exercised for 3 half hour periods and observations continued for 2 hourly post-work controls. The 1st day of fasting there was no increase in excreted nitrogen during exercise. The 2nd day, there was some increase during exercise, a maximum increase on the 4th day, and, less each succeeding day, until there was no increase on the 8th, 9th and 10th days. The increased nitrogen, during the working period, continued during the post-work hours, *i. e.,* on the 4th day the preliminary period averaged 128 mg. of urinary nitrogen per hour, the exercise period 180 mg., and the post-work period 180 mg. After giving 500 grams of sucrose on the 11th day of fasting, exercise on the 12th day produced no increase in nitrogen metabolism.

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* Fellow in Medicine, National Research Council, 1924-26.

Our insulin results indicate that the increased nitrogen excretion, after insulin, is correlated with the presence of carbohydrate reserves in the body rather than with a calling forth of reserve protein to counteract the insulin after the depletion of the carbohydrate stores. However, the exercise experiments show that muscular work can be accomplished without an increase in nitrogen excretion if sufficient carbohydrate is available. This is also the case after about 8 days of fasting and exercise. Exercise is accompanied by an increased protein metabolism during the early part of the fast.

3281

Experiments on Activation of Cholesterol Derivatives and Allied Sterols by Ultra-violet Irradiation.

ALFRED F. HESS AND A. WINDAUS.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Chemical Laboratory, University of Goettingen, Germany.

It has been shown repeatedly that cholesterol can be rendered antirachitic by subjection to ultra-violet rays. In a previous paper[1] it has been pointed out that "the saturated reduction products of cholesterol and phytosterol—dihydrocholesterol and dihydrophytosterol—were not activated by irradiation and did not acquire antirachitic properties." It was further shown that "cholesterol acetate, an unsaturated ester of cholesterol, developed antirachitic potency as the result of irradiation."[2] These results were "regarded as additional evidence that the double bond plays an essential rôle in activation." It has recently been shown by Rosenheim and Webster that in addition to the unsaturated carbon linkage of the sterol molecule, the secondary alcohol group is intimately concerned in the activation by ultra-violet rays.[3]

The present communication concerns itself with the effect of irradiation on a large number of cholesterol derivatives prepared by one of us (Windaus). The following derivatives were tested: B-cholesterol, hetero-cholesterol, α-phytosterol; cholesteryl-chloride, cholesteryl-amin-acetate; cholesten, pseudo-cholesten, cholesterilen, cholestan-4-7 diol, cholestan 4-on 7-ol, cholestenon; stigmasterol, α-amyrol, cholesteryl-ether, cholesterol-dibromide, cholesterol-ozonide (non-irradiated and irradiated), apocholic acid.

These preparations were made with the idea of noting what rearrangement or substitution in the molecule could be made without interfering with the specific action of irradiation. Feeding tests on rats showed that none of these derivatives had acquired antirachitic potency. This result is of interest in view of the slight degree of deviation from cholesterol which has been made in some of the derivatives. Attention may be called in this connection particularly to B-cholesterol, in which there is a very slight rearrangement in the secondary alcohol, and the hetero-cholesterol, which is characterized by a shifting of the double bond. It is also of interest to note, that although it had been found previously by one of us, that cholesterol acetate, an unsaturated ester of cholesterol, developed antirachitic potency as the result of irradiation, the amin-acetate failed to become activated.

A detailed discussion of the significance of these tests of various cholesterol derivatives and allied sterols will be reported elsewhere.

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[1] Hess, A. F., and Weinstock, M., *J. Biol. Chem.*, 1925, lxiv, 181.

[2] Hess, A. F., Weinstock, M., and Sherman, E., *J. Biol. Chem.*, 1926, lxvii, 413.

[3] Rosenheim, O., and Webster, T. A., *Biochem. J.*, 1926, xx, 537.

3282

The Anti-Bacterial Activity of Egg-White.

ALEXANDER SANDOW. (Introduced by Calvin B. Coulter.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

The utilization of egg-white as pabulum by bacteria is a fact of common experience. Egg-white also possesses marked anti-bacterial properties for certain bacteria. This phenomena was investigated in the work reported here.

Egg-white was removed aseptically from fresh store eggs with a sterile syringe and serially diluted with meat-infusion broth pH 7.5 to 7.6. One cc. quantities of these dilutions were distributed into separate sterile small (Wassermann) test tubes and each tube, including a control tube of broth, was inoculated and well mixed with 0.1 cc. of a 24-hour broth culture of the organisms used. Similar series were made with inocula of 0.1 cc. of a 1-100 and 0.1 cc. of a 1-10,000 dilution in broth of the broth cultures. All tubes were incubated for 20 to 24 hours and then observed for inhibition of growth. In addition, loopfuls of the mixture from each tube were separately streaked out on agar plates (blood agar for streptococcus) which were incubated for 24 hours and then examined for growth. Absence of growth in the streaks on the agar plates was assumed to indicate sterilization of the inocula in the corresponding tubes.

With this technique the following results were obtained:

B. typhosus, B. coli, V. cholerae and *B. proteus* were unaffected by even undiluted egg-white. Slight inhibition of growth was observed in tubes containing undiluted to 1-8 egg-white, but this may be due to the relative absence of the nutriment of the broth.

B. subtilis, Staphylococcus albus and *Streptococcus hemolyticus* ("H" strain, Gay) were definitely inhibited from developing in dilutions of egg-white up to 1-8 and were killed by whole and 1-2 egg-white. These results were more clear cut with the smaller inocula.

Sarcina "A" (a strain isolated from the laboratory air) was inhibited from growing in dilutions of egg-white up to 1-12800 and was killed and dissolved by dilutions up to 1-6400.

These results practically wholly confirm previous work on this subject by Wurtz,[1] Laschtschenko,[2] Sperry and Rettger,[3] Gay and Morrison,[4] and Fleming and Allison.[5]

Native egg-white is quite alkaline, usually about pH 9.2 to 9.4. When diluted in broth at pH 7.5 to 7.6, as in the described experiments, the pH of the dilutions varied with the relative amounts of egg-white and broth, decreasing to the pH of the broth as a limit with the increase in dilution of the egg-white. The pH of the mixtures ceased to vary in the dilutions 1-32 or 1-64 and greater.

This factor cannot be controlled by simply adjusting egg-white to the desired pH and diluting with broth at the same pH. Such adjusted dilutions, when containing relatively large amounts of egg-white, tend to return to the original pH of the egg-white. This is probably due entirely to the slow liberation of CO_2 from the egg-white to which acid has been added.

To control the pH factor completely when using dilutions containing large amounts of egg-white it was necessary to carry out the tests in very small test tubes tightly stoppered with sterile corks. The small free volume, above the test mixtures in such stoppered test-tubes, soon comes into equilibrium with the egg-white-broth dilution with respect to the carbonate-bicarbonate buffer system and the pH of the mixture remains constant.

B. typhosus, B. coli, V. cholerae and *B. proteus* have been only cursorily examined with this technique. The results indicate, however, that egg-white at different pH's has no effect on these organisms.

Detailed experiments on the anti-bacterial power of egg-white at different pH's on *Streptococcus hemolyticus* show that at pH 9.2 a dilution of 1-4 or 1-8 egg-white in broth is bactericidal and probably bacteriolytic for this organism. At pH 7.6 or pH 6.0, however, even undiluted egg-white serves as an excellent culture medium for *Streptococcus hemolyticus* "H".

In view of the extremely marked bactericidal and bacteriolytic properties of egg-white against sarcina "A" it was considered worth while to investigate more closely this phase of the problem. If an opaque suspension of this organism is mixed with a solution of egg-white whose final concentration is 1-100 and the mixture kept at 37° C. the following changes are observed: the mixture gradually becomes transparent, the color becomes yellow, the viscosity increases, the pH decreases, in 10 to 15 minutes the organisms are apparently entirely killed and dissolved, and, at the end of this time a yellow amorphous substance can be precipitated out of the solution by adding acid to a certain pH. Microscopic examination of such a test shows that the bacteria disintegrate and dissolve in a manner similar to that of the Pfeiffer phenomenon.

Further tests with sarcina "A" were carried out with a technique similar to that already described except that peptone water was used instead of meat-infusion broth, and a standard suspension of cells from a washed agar slant, in place of a broth culture. In view of the high dilutions of egg-white giving significant results it was not necessary to use the small stoppered test tubes.

Results were read in terms of the dilution of egg-white causing complete lysis of the inoculated cells after 24 hours incubation. Complete lysis was always accompanied by sterilization of the inoculum.

The thermolability of the anti-bacterial principle for sarcina "A" was investigated by heating at 65° C., 1-20 dilutions of egg-white in distilled water at different pH's. After definite intervals of time samples from each tube were removed and tested for the presence of the anti-bacterial principle. At pH 8.4 it was destroyed in 15 minutes; at pH 6.8 in 30 minutes; at pH 5.0 it was not destroyed after 60 minutes.

The effect of the concentration of salt (NaCl) on the lysis of sarcina "A" was studied by substituting different salt solutions for peptone water as the egg-white diluent. It was found that the optimum salt concentration was 0.145 N, that is, isotonic, in which the greatest dilution causing complete lysis was 1-6400. In distilled water, and isotonic saccharose solutions as well, the lytic action was suppressed to one-eighth this value; and in 0.435 N NaCl, to one-sixty-fourth.

The pH of the peptone water-egg-white dilutions was found to have a marked influence on the lysis of the sarcina organism. The peptone water was easily adjusted to different pH's by the addition of small amounts of acid and alkali. The optimum was about pH 6.0, at which a 1-12800 to 1-25600 dilution of egg-white caused complete lysis. On the acid side of this optimum pH the activity was very sharply suppressed, whereas on the alkaline side, the drop in activity was more gradual. It will be recalled that the action of egg-white against *Streptococcus hemolyticus* took place at pH 9.2 and not at all at pH 7.6 or 6.0.

The work on this problem to date has been done with chemically unaltered egg-white. Experiments are now in progress designed to isolate and purify the anti-bacterial principle and then to examine more closely its nature and activity.

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3283

Respiration of So-Called Filterable Viruses.

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Some time ago one of us described a simple micro-respirometer which permits detection of very small amounts of CO_2.[1]

By the use of this method we were able to detect production of one cubic millimeter of CO_2 by 15 million staphylococci in less than ten minutes. However, this is not the limit of the sensitiveness of this method, since the time during which measurable amounts of CO_2 may be permitted to accumulate in the respirometer may be extended for days, so that the negative findings obtained by this method are significant.

Having failed to detect by this method evidence of respiration in filtrates of so-called bacteriophage,[1, 2] we thought it of interest to inquire into the question of respiration (CO_2 production) by other so-called filterable viruses, particularly those of rabies and herpes. Since these viruses cannot be secured free from tissue cells, it was necessary, at the outset, to differentiate, on the one hand, between the oxygen uptake and the respiration proper with production of CO_2, and, on the other hand, between the respiration of tissues as against that of the viruses themselves. The parallel use of Warburg's respirometer to determine oxygen uptake[3] and of our closed cell respirometer[1] gave us the means of fulfilling the first requirement. In order to eliminate the effect of respiration of surviving tissue cells, fresh autopsy material was placed into 50 per cent glycerine and preserved in this condition for months. From time to time samples of these tissues were emulsified and subjected to test. In all, 6 experiments were made with fixed virus and 15 with virus of herpes. Emulsions, made from preserved tissues during the first 8 weeks of ageing in glycerin, showed oxygen uptake and production of CO_2 at approximately the same rate as did normal brain tissue preserved for the same length of time and under similar conditions.

After 3 months of ageing the fixed virus took up oxygen and transmitted rabies to fresh animals, but no longer produced CO_2. When emulsified in Ringer's solution containing 1 per cent phenol, and kept at room temperature for 24 hours in the absence of oxygen, it continued taking up oxygen, when transferred to the respirometer, at approximately the same rate as before exposure to phenol, although it had completely lost its infectivity by this treatment. It was evident, therefore, that oxygen uptake could not be attributable to the presence of the virus.

After a subsequent period of 3 months of ageing in glycerin, the samples of tissues yielded emulsions which were still infective but produced no detectable CO_2 during 48 hours sojourn in the respirometer, either under aerobic or anaerobic conditions.

The emulsions tested contained in each case 0.05 grams of tissue (by moist weight). This is twice the amount used for intracerebral injection in the test animals. Twenty-five thousandths of a gram of this material invariably produced typical symptoms, in a minimum number of days, and therefore, presumably contained a large amount of active virus.

These experiments suggest that either the viruses studied do not respire, or that the respiration proceeds at such a low rate that its detection is beyond the limits of the sensitiveness of the method.

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3284

Photopharmacology. VII: Effect of Ultra-violet Rays on Germicidal Properties of Mercurochrome.

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The effect of Ultra-violet radiation on the potency of Mercurochrome Solution was studied on *B. coli*. The method used was as follows: 2 cc. of Mercurochrome Solution 1:500 was inoculated with one standard loopful of a twenty-four hour culture of *B. coli*. At the end of the exposure time, 0.1 cc. of the drug-and-organism mixture was removed by means of a sterile pipette and transferred to 5 cc. of sterile broth. Inasmuch as controls showed that in this transfer enough drug was carried over to prevent the growth, a second transfer of 1 cc. was made to 9 cc. of sterile broth. A control of this latter method of dilution showed that prompt and heavy growth of bacteria was obtained with these dilutions in the absence of the drug, while the amount of drug present in the second transfer tube was not sufficient to visibly inhibit the growth of the organisms. The times of exposure which were studied were 1, 2, 3, 4, and 5 minutes.

The irradiation of Mercurochrome Solution was performed in the following ways: Solution of Mercurochrome were radiated with the Hanovia Alpine Sun Lamp at a distance of 30 centimeters. Other samples of the same solution were radiated with a water cooled Krohmayer Lamp (Hanovia Company) at a distance of 5 centimeters from the mercury arc. The solutions were exposed to the Ultra-violet rays in three different containers, namely, 1. Quartz Test Tube; 2. Pyrex Test Tube; 3. Ordinary Soft Glass Test Tube. The rays emitted by the Krohmayer Lamp ranged as low as 2000 Angstrom units, while those emitted by the Alpine Sun Lamp at close range extended a little farther, namely, to 1850 Angstrom units. Spectroscopical examination of light transmitted by the Pyrex tube indicated that the shortest weight transmitted by them were 2820 Angstrom units, while the shortest wave lengths transmitted by the ordinary glass tube were not shorter than 3000 Angstrom units.

Two sets of experiments were performed. In the first series the exposure of all the solutions in the different test tubes and with both lamps was for 30 minutes. In the second series of experiments a longer exposure, namely, 1 hour and 15 minutes, was made. The results obtained may be summarized briefly as follows: No visible change in the color of the solution or any precipitation was observed. Bacteriological examination, carefully performed according to the method described above, showed that no deterioration was produced by the irradiation in the antiseptic or germicidal potency of the drug.

3285

Effect on CO_2-combining Power of Direct Irradiation of Blood in vivo.*

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In previously published papers, a method was described for direct irradiation, with a special carbon arc, of circulating blood in etherized dogs; also some effects on blood pressure[1] and various blood constituents and properties.[2, 3, 4] Since no adequate explanation has been found for most of the results reported, it has seemed advisable to investigate the possibility of other changes occurring, under the experimental conditions described, which might add to our understanding of photobiological effects.

It is the purpose of this paper to report the results of an investigation of the effect on carbon dioxide combining power of the blood of dogs subjected to the same technic as those used in the other investigations. C. Kroetz[5] has reported that ultra-violet and Roentgen irradiation produce immediately a temporary acidosis, with reversal of the effect after a few hours. It has been generally observed that ether anesthesia decreases the CO_2-combining power of the animal's blood.[6, 7]

It has also been noted that injection of medium doses of morphine, subsequent to complete ether anesthesia, will again increase this factor.[6, 8] It does not fall within the province of this paper to discuss the underlying causes of these changes, but to report our results for the sake of a complete picture of the immediate changes resulting from this method of irradiation.

A series of control experiments was made, in each of which a sample of blood was drawn from the saphenous vein, after which the animal was quickly etherized and subjected to all the operative technic previously described but without radiation. Further samples were drawn at varying intervals and the total amount of CO_2 in solution determined by the method of Van Slyke.[9] In all cases the blood was heparinized *in vivo*. Each determination was made in duplicate. The results are shown in Table I.

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* A part of the expense of this investigation was borne by a grant from the Research Committee of the American Association for the Advancement of Science, for which grateful appreciation is extended.

TABLE I.

CO_2 per 100 cc. of blood. Control series. Time in five minute intervals.

No.	Min.	5	10	15	20	30	35	40	45	50	60	65	70	75	80	85	90	95	100	105	110	115	120
1	54.6		46.2							44.6		38.5			36.4			37.3			36.4	36.5	
2	42.4		31.0		31.4	35.1		29.8		29.9	31.0		26.3				29.6				30.0		
3	51.6	41.8	34.4		34.4			34.7		32.0													
4	51.0	45.5	47.9	47.1						44.9							36.1						
5	60.0	53.9	56.5	55.6						53.3							43.6						
6	44.0	36.8	36.8		37.0		40.5				34.4						37.3						
7	51.1	39.8	39.9					40.2					39.8		39.7	40.3							
8	40.8	36.5	36.5					36.5				34.9		36.2	35.0		35.8						
9	50.0				43.1					34.4			30.3				27.8		28.2		28.2		28.2
10	53.1			46.5					54.7			42.4		43.0									

TABLE II.

Experimental series. Irradiation begun at *.

No.	Min.	10	15	20	25	30	40	45	50	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	
1	55.4	37.0	*						40.5					40.5			40.5		39.8							
2	47.1			42.8	*		40.1		40.1					40.0					[illegible]	38.4		38.8				
3	59.1		45.7				*		45.5					45.9						43.0			42.9		34.7	
4	48.9		40.8				*	43.1					42.6						34.9			30.5		34.6		
5	52.1		46.1					*		36.6						34.9						34.3			28.2	
6	54.7					42.8	*				42.8					[illegible]	47.9			47.6			46.6			
7	56.3	45.2	*		*		36.9					34.9						32.2			27.5					
8	50.6				38.3											37.3			36.8	37.5	36.7	29.4				
9	48.4		41.1	*		40.9							37.1		33.6			31.5			29.6					
10	55.5			41.4	*			33.6					33.7			30.4			29.2							

* Irradiation begun.

TABLE III.

Control series. Morphine injection at m.

No.	Min.	10	15	20	30	40	45	50	55	60	65	70	75	85	90	95	100	110	115	120	130	135	150
1	61.7		38.2	m		40.2						41.9		42.1			42.2		41.0		43.0		
2	55.9		43.0	m	52.4		54.2			54.5					51.4			47.7		50.7			
3	—		38.5	m			35.0		35.2		35.0		35.7	36.4		34.5		34.6					
4	55.9	m	44.8					46.8					45.8		48.1		48.9	48.5		48.2			
5	47.9	m	44.3					43.7					43.2		39.9		39.3	39.0		39.2			
6	55.8		41.3							41.6			31.3		31.6		31.5	m				31.5	31.0

TABLE IV.

Experimental series. Morphine at m. Irradiation at *.

No.	Min.	10	15	20	25	30	35	40	45	50	55	60	75	85	90	100	105	110	115	120	125
1	52.8		m	38.9				*			42.1			38.4							27.3
2	58.1		41.2	m		*			40.8			40.1	37.4		35.4		35.2			38.4	
3	49.2		39.3		m	*				37.8			39.8				42.1		40.3		42.6
4	51.9	m	44.0		*					50.4			52.1		57.0	56.9		54.5		55.3	
5	54.6	m	38.4	*						25.8			23.6		26.7						
6	53.3	m	42.7	*						43.6			43.9		43.7	44.1		43.2		37.2	
7	55.5	m	49.7	*			35.6						47.1		45.5	47.9		50.4		50.7	
8	47.5	m	36.2	*						36.9			38.9		33.6	35.6		28.0		32.0	

In a second series, similarly managed, the blood was irradiated *in vivo*. For the sake of comparison with the results already reported, blood pressure was recorded in all series. No correlation was found possible between changes in blood pressure level and CO_2-combining power. The results of this series are shown in Table II.

Since there could be shown no evidence of any constant effect of irradiation, it was decided to determine whether any effect could be found after the application of morphine, since this would increase the CO_2, after it had been decreased by ether. A control series was undertaken in which each animal, after complete ether anesthesia, and after all details of operative technic were completed, received a subcutaneous injection of 25 mg. of morphine sulphate per kilo of body weight. The first sample of blood was drawn before anesthesia, the second, just before morphine was administered, and subsequent samples at varying intervals as shown in Table III.

Another series prepared as above was subjected to irradiation after morphine injection. (Table IV.) As in the case of the first procedure, it was not found possible to demonstrate any constant changes in the CO_2-combining power, during or after irradiation. Nor was it found possible to correlate any observed variations with any other factor, although blood pressure was invariably depressed to a marked degree in both series of irradiations, as already reported,[1] even though considerable depression had already resulted from the morphine.

The results shown in Tables I and III, in general, confirm the findings of others with regard to the effects of ether anesthesia, and, of morphine and ether combined. With the single exception of number 6, Table II, there was a progressive decrease throughout each experiment in series I and II, which was more rapid in the earlier stages. While subject to some fluctuations during later stages, in both control and experimental series, there were no significant variations that could be attributed to irradiation.

Dog number 5, Table IV, died suddenly at the end of 90 minutes and after 70 minutes of irradiation. Such a result was not uncommon, in the previously reported experiments, and, seemed to be due to irradiation of an animal that, for some reason not understood, was extraordinarily sensitive, since the degree of anesthesia was, in no case, such as to warrant expectation of any such results.

Administration of morphine usually, though not invariably, caused an increase of CO_2, but the failure to do so was as frequently apparent in the control series as in the experimental series, so that this result does not appear to be due to irradiation.

In view of the frequently reported effects of various types of irradiation on general metabolism, the failure to find any constant changes in CO_2-combining power in these experiments is disappointing. But so far as our results are concerned, it can be said that irradiation of blood *in vivo,* by our technic, does not produce any constant effect on this factor, at least within relatively short periods.

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3286

Accessory Etiologic Factors of Pneumonia in Rabbits. I: Effect of Putting Brilliant Green into Nasal Passages.

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A weak solution (from 0.1 to 0.3 per cent) of brilliant green dropped into the nostrils of rabbits carrying *B. lepisepticum* incites severe snuffles and pneumonia. In rabbits not carrying the organism naturally, the same effect can be obtained by putting a small amount of culture into the nostrils before the dye is given or 1 or 2 days afterwards. The dye alone has little or no visible effect.

Thirty-two rabbits having very slight serous discharges from the nose, and, a positive lepisepticum culture, were given 0.2 cc. of the solution (0.3 per cent) in each nostril. All of the rabbits developed very profuse nasal discharges, which often caused such obstruction of the nasal passages, that they were forced to breathe through the mouth. To all intents and purposes the rabbits had a very severe and active form of snuffles. Twenty-one of them developed pulmonary infections and died from the 3d to the 15th day after treatment, the average length of life after treatment being 8 days. The others gradually improved after a prolonged siege of snuffles.

The more acutely fatal cases had typical lobar pneumonia, one or more lobes being in the state of complete red hepatization. The other lobes had either a normal appearance or showed some injection, edema or emphysema. Those living longer usually had a general fibrino-purulent pleurisy and pericarditis, and less extensive solidification of the lungs but more pronounced edema and emphysema.

Six rabbits having negative cultures, *i. e.,* negative for *B. lepisepticum,* were given nasal inoculations. Five days later 4 of them had a light form of snuffles with serous discharges and 2 had rather profuse mucous discharges. All gave positive lepisepticum cultures. Brilliant green (0.1 per cent) was dropped into the nostrils of each rabbit. All of them developed severe snuffles and 2 died of pneumonia, 1 on the 3d day and 1 on the 13th day.

In another experiment a litter of 6 rabbits was used. They were free of *B. lepisepticum.* Brilliant green (0.1 per cent) was dropped into the nostrils of 4 of them. On the following day there was no effect of the brilliant green to be seen. All 6 of the rabbits were now inoculated intranasally with *B. lepisepticum.* The 4 having had the brilliant green developed severe snuffles and 3 died of pneumonia, on the 4th, 8th, and 9th days respectively. The 2 not having had brilliant green had a light form of snuffles and had "clean noses" at the end of 2 weeks but were still carrying the organisms.

3287

A Low Resistance Air Valve.

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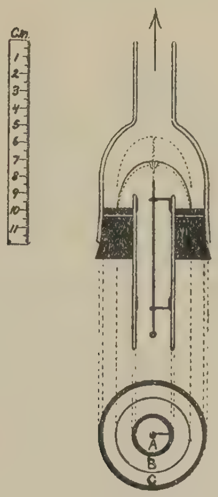
The valve is particularly adapted for respiratory work. It consists of a vertical 9 cm. length of 22 mm. glass tubing; the tube projects 1.5 cm. through the center of an inverted No. 13 rubber stopper. The upper surface of the stopper has a layer of canton flannel. A light celluloid cup, in cross section a parabola, and 4.5 cm.

in diameter, covers the open tube and rests on the flannel. Projecting downward from the center of the cup is a light rod of pyrex glass, which passes through 2 gold bearings. A check on the end of the rod permits it to move 1.5 cm. vertically. The stopper fits into an inverted glass bell 7 cm. high. At the top, the bell is continuous with a 5 cm. length of 22 mm. glass tubing. A layer of mercury 0.5 cm. deep is placed on the flannel surface. The celluloid cup floats on the mercury. When suction is applied at the upper tube, the cup rises vertically from the mercury and permits the incoming air to pass; when pressure is applied the cup is forced down to the flannel surface and the mercury seals the joint. When used as an expiratory valve, the condensed water is drained off by a small wick which extends through the stopper at the periphery.



SCIENTIFIC PROCEEDINGS.

Peking Branch.

Peking Union Medical College, China, October 25, 1926.

3288

Relation of the Reticulo-Endothelial System to the Formation of Amyloid.

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In a recent article Domagk[1] states that he succeeded in producing amyloid in the spleen and liver within 10 minutes, by intravenous injection of a large quantity of living cocci into a normal mouse. Furthermore, that he could produce amyloid within 2 minutes in the spleen and liver of a mouse, which had several injections of dead cocci previous to the final intravenous injection of living cocci. He also stated that this amyloid is formed by the phagocytic endothelial cells, and describes changes in these cells before and during the appearance of amyloid in their immediate neighborhood. This amyloid did not react to any of the specific stains (gentian violet or iodine), but showed metachromasia when stained with cresyl violet and giemsa.

In repeating these experiments we injected congo red intravenously shortly before killing the animals, in order to mark out the amyloid. In previous experiments this vital staining has proven to be the most delicate and reliable method for demonstrating the earliest traces of amyloid.[2]

We failed to find any traces of amyloid with this method in two series of animals, each consisting of 10 mice.

Many morphological pictures seen in experimental amyloidosis are inconsistent with the idea that amyloid is formed by the precipitation of a substance circulating in the blood stream. They suggest rather its local formation. Experiments were therefore undertaken to find any possible relation between Reticulo-Endothelial Cells and amyloid.

Amyloid was produced in mice by daily injections of a 5 per cent solution of nutrose, as described by Kuczynski,[3] and the R. E. C.* were marked out by an intravenous injection of India ink before starting the daily injections of nutrose.

In studying the mutual relations between amyloid and R. E. C., this substance was found to be located exactly in those places where R. E. C. are most abundant: around the follicles of the spleen, around the periportal veins, and in the intermediate zone of the liver lobules, in the glomeruli, in the tissue between the tubules of the kidneys, and in the intestinal villi.

It also was found that amyloid invariably appears first in the form of small isolated patches, which suggests its local formation. This patchy formation of amyloid, and its presence in the immediate neighborhood of the R. E. C., marked out by India ink or trypan blue, supports the idea that it might be formed, actively, in some way, by these cells. Small patches of amyloid in the intermediate zone of the liver lobules are almost always surrounded by cells containing ink, which separate the amyloid from the liver cells, and which make these patches appear to be in the lumina of capillaries. Since R. E. C. are located in the capillaries, these pictures also support the idea that R. E. C. may in some way be responsible for the presence of amyloid. Otherwise amyloid, were its position outside the wall of capillaries, could not be surrounded by R. E. C., which contain ink injected several weeks previously.

Commonly one sees separate contiguous small patches of amyloid surrounded by liver cells. Cells which contain ink granules separate these patches from the liver cells and from each other. This seems to indicate the mechanism by which contiguous streaks of this material are formed. This contiguity is seen in advanced cases.

Certain morphological changes in R. E. C., before and during periods of amyloid, suggest severe damage of these cells. It is possible that amyloid is formed only by damaged or altered cells.

Organs of mice, in which India ink was injected intravenously, after amyloid was already developed, showed a marked decrease, and even absence of ink-containing cells in areas where amyloid was present. Identical pictures were seen in organs of mice injected repeatedly with trypan blue after the development of amyloid.

These findings indicate that there are either no R. E. C. in these areas, or that these cells have been markedly altered physiologically. Other parts of the liver and spleen, where there was no amyloid present, showed the usual amount of phagocytic cells loaded with India ink.

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* Reticulo Endothelial Cells.

There is no evidence that R. E. C. are primarily converted into amyloid. Were this true, the patches of amyloid would constantly contain large numbers of ink granules. This, however, is not the case. R. E. C. are secondarily involved in this process; they become partly and completely surrounded by amyloid and are found embedded in this substance. Finally, they die leaving only their phagocytized ink granules in their places.

In order to determine whether it is possible to influence the time of the appearance of amyloid by blocking the R. E. C. with India ink, series of mice were repeatedly injected intravenously with India ink, while amyloid was produced by subcutaneous injections of nutrose. Control animals were injected with nutrose only. In 5 out of 6 series the appearance of amyloid was markedly delayed in mice whose R. E. C. were filled with India ink. In one series, animals with and without ink showed amyloid at the same time; in this series the animals had received only one intravenous injection of India ink.

It is true that these experiments have only limited value. In the first place it is impossible to block the R. E. C. completely; secondly, there is considerable irregularity in the time necessary for the experimental production of amyloid, even with standardized injections of nutrose. But since the attempted blockade of R. E. C. did result in delay of the appearance of amyloid, this fact, though in itself inconclusive, at least supports the other observations leading to the idea that R. E. C. are in some way concerned in the formation of amyloid.

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3289

The Nutritive Value of the Mung Bean, Phaseolus Aureus Roxburgh.

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The mung bean prepared in various forms, constitutes an important part of the Chinese dietary. The commonest variety, one which was employed in this investigation, is the olive green bean, not the less common yellow seed described by Roxburgh. The air-dried bean is ovoid to globose in shape and about 4.5 mm. in length. According to Embrey and Wang[1] the mung bean has the following percentage composition: water 11.86, protein 22.10, carbohydrate 58.80, fat 0.80, fiber 3.09, and ash 3.55.

Embrey[2] has reported feeding experiments on the mung bean, employing Chinese white mice as the experimental animals. The results, however, were unsatisfactory. As experimental animals, Chinese white mice had not been properly standardized. The animals were extremely small, the adults weighing not more than 13 grams, and their growth capacity was limited.

Feeding experiments on a standard animal, namely, the albino rat *(Mus norvegicus albinus)*, have shown the following results.

With the intake at about 18 per cent of the ration, and representing not less than 20 per cent of the total calories, the proteins of the Chinese mung bean are biologically complete.

The mineral content of the mung bean is deficient apparently chiefly in Ca and Cl.

The mung beans, constituting 60 per cent of the diet, furnish a moderate amount of vitamin A. At 30 per cent level they are distinctly deficient in this vitamin.

The mung beans are a source of vitamin B. At the level of 30 per cent of the food mixture they furnish sufficient vitamin B for the normal nutrition of the rats.

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3290

The Effect of Insulin on Blood Cholesterol, Fat and Sugar in Nephrosis.

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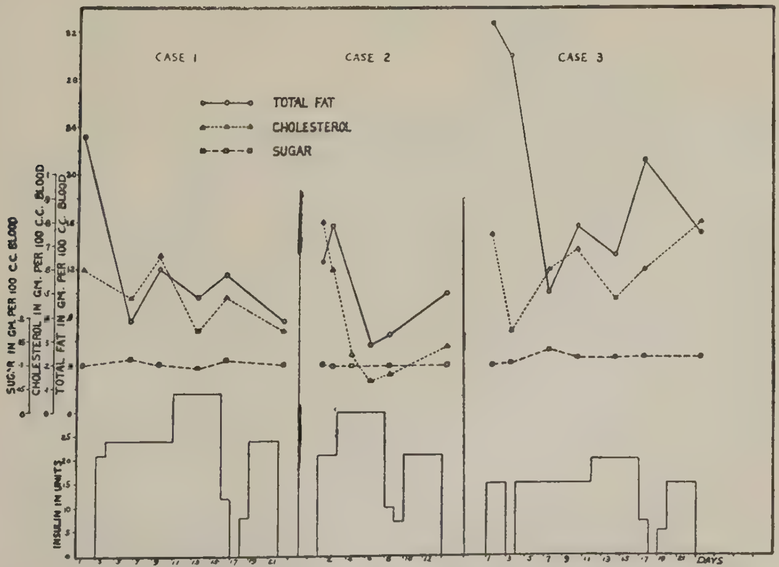
It is well known that in severe diabetes, blood fat and cholesterol are often increased, and the lipemia in such cases disappear promptly with insulin treatment. In nephrosis, there is also marked hypercholesterolemia and lipemia, but normal blood sugar. Observations on the effect of insulin on the blood fat and cholesterol in such condition, however, have not been recorded as far as we can ascertain. It is the purpose of the present communication to report 3 cases of nephrosis in which frequent observations were made of the blood fat, cholesterol and sugar after the administration of insulin, in varying doses, three times daily before meals, for certain periods of time. The results obtained are summarized in the chart.

In each of the 3 cases, injection of insulin was followed by a decrease of both cholesterol and fat in the blood. The decrease of cholesterol was immediate, as shown in cases 2 and 3, in which blood was taken on the 1st or 2nd day after the injection, while



THE EFFECT OF INSULIN ON BLOOD CHOLESTEROL, FAT AND SUGAR IN NEPHROSIS.

the decrease of fat, though more extensive (cases 1 and 3), lagged behind. On continuing the insulin administration with the same dosage, the initial fall of cholesterol and fat was followed by a tendency to rise in both of these elements, though not to the original level. In cases 1 and 3, when the insulin was increased on the 11th and 12th day respectively, a second decrease of both cholesterol and fat took place, but they again tended to increase upon the continuance of even larger dosage of insulin. After insulin was withheld for a day or two (cases 1 and 3), resumption of the drug could again lower the cholesterol and fat content of the blood (though the last determination of cholesterol in case 3 was unaccountably high).

The blood sugar, on the other hand, showed practically no change in spite of the continued insulin administration. This raises the question in our minds whether the presence of excessive cholesterol and fat inhibits the action of insulin on carbohydrate metabolism.

With the insulin therapy, all the three patients felt better subjectively, with increased appetite and brightening of mentality. There was, however, no increased diuresis or decreased albuminuria noticed in these cases.

During the course of this investigation, Mahler's work[1] appeared. He demonstrated a slight increase of blood cholesterol, along with the increase of blood sugar during ether anesthesia. This cholesterol elevation could be prevented by previous insulin injection, thus substantiating our conclusions concerning the depressant effect of insulin on blood cholesterol.

From our work no conclusion can be drawn in regard to the fate of cholesterol and fat after they are made to disappear from the blood by insulin. Nitzescu and Cadariu[2] found that insulin increases cholesterol and fat deposition in the liver and lungs of dogs, and depancreatized animals are unable to store up cholesterol and fat in these tissues. The insulin action in nephrosis may well be a matter of increased storage, but the possibilities of increased output and increased utilization are not excluded. We are continuing our work on that line.

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[1] Mahler, A., *J. Biol. Chem.*, 1926, lxix, 653.

[2] Nitzescu and Cadariu, *Compt. rend. Soc. de Biol.*, 1925, xcii, 1503.

3291

Vital Staining Characteristics of the Epithelioid Cell in Experimental Tuberculosis.

J. R. CASH.

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Twenty young rabbits, weighing from 600 to 800 grams, were each inoculated intravenously with 1/2 cc. of saline emulsion of a three weeks culture of virulent bovine tubercle bacilli. The emulsion contained about 35 bacilli to each oil immersion field. After 10 days, animals from this series were studied at bi-weekly intervals, to 6 weeks after infection, in the following manner:

Ten to 15 cc. of a 1 per cent normal saline solution of neutral red* was slowly injected into the ear vein. After 10 minutes the rabbit was quickly killed by air embolism. Thin slices of the lungs, which in most cases were well stained with neutral red, were fixed in Zenker-formol for 12 to 24 hours. Thin blocks of tissue (2 mm.) were quickly dehydrated, without washing, in acetone, cleared in benzene or xylol, and rapidly imbedded in paraffin.

Thin sections are then made and stained with carbol fuchsin and methylene blue. In the staining process both alcohol and water should be avoided as much as possible. The paraffin is removed by xylol, and the section transferred to acetone. It may then be passed rapidly through water, and washed quickly in Gram's iodine, or Lugol's solution, to remove most of the mercurial precipitate. After blotting dry, the staining with carbol fuchsin was performed on a warm plate in the usual manner. This process should not exceed more than one minute. This allows for rapid decolorization in acid alcohol which is essential, if the color of the neutral red in the section is to be preserved. The acid alcohol at the same time removes the iodine from the tissues. After quickly rinsing in water, the section is blotted dry, stained with Loeffler's methylene blue, decolorized slightly in 95 per cent alcohol, dehydrated further in acetone, cleared in xylol and mounted in balsam. In properly stained sections the neutral red taken up by cells in the lung tissue does not vary appreciably in color and amount from that in fresh, unfixed films from the cut surface of the lung, or unstained sections of fixed tissue. One experiences no difficulty in identifying the tubercle bacilli which are stained brilliant scarlet; the neutral red is of rather salmon-red color.

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* National Aniline and Chemical Co.

In such preparations neutral red is seen in alveolar epithelium, in large phagocytic cells of two varieties, and in the epithelioid cells of the tubercles. Very little of the dye is taken up by the alveolar epithelium, which may be readily distinguished. The first and less numerous group of vitally stained, mononuclear phagocytes consists of large, irregularly-shaped cells with small nuclei, which are often obscured by masses of dye or cellular débris within the abundant cytoplasm. Such cells are found mainly in the alveolar walls, interlobular septa, and at times within the alveolar spaces. They seem in no way directly or specifically related to the tuberculous lesions, and have never been seen to contain tubercle bacilli.

The other group of vitally stained phagocytes consists of large round or oval cells, with abundant cytoplasm, and large, sharply defarcated nuclei. The nuclei, in sections, vary in shape with the direction in which the cell is cut, being round, oval, or indented, and have a light violet color when stained with methylene blue. The cytoplasm is just perceptibly stained pale blue, and a large zone by the side of the nucleus is not stained at all. In this clear zone the neutral red is taken up in coarse granules of about the same size, which are arranged about a small clear space in the form of a rosette. These cells are found in small groups within the alveolar walls, septa and alveoli. Moreover, they prove to be the large mononuclear cells with which the alveoli are filled in patches of tuberculous pneumonia. They frequently contain tubercle bacilli.

The epithelioid cells of the young tubercles have identically the same staining characteristics as this latter group of mononuclear cells. Due to their more compact arrangement, the shape of the epithelioid cells is often more elongated or irregular, but otherwise these two groups of cells show no fundamental differences. The epithelioid cells contain many tubercle bacilli, and in each cell, cut through or near the nucleus, one sees the characteristic rosette of neutral red. One frequently sees epithelioid cells with two or more nuclei grouped about a common rosette of neutral red. In several instances giant cells with 10 to 15 nuclei surrounding a large, single rosette were found.

It will be recognized at once that this characteristic staining with neutral red of the epithelioid cells of tuberculosis, and the mononuclear cells of tuberculous pneumonia, is the same as that which has been described for the large mononuclears or monocytes of the peripheral blood.[1] The following table summarizes the results of differential blood counts, by the supravital technique, upon 10 of the rabbits of this series. It confirms the numerous other observations of an increase in the monocytes of the blood during tuberculous infection.

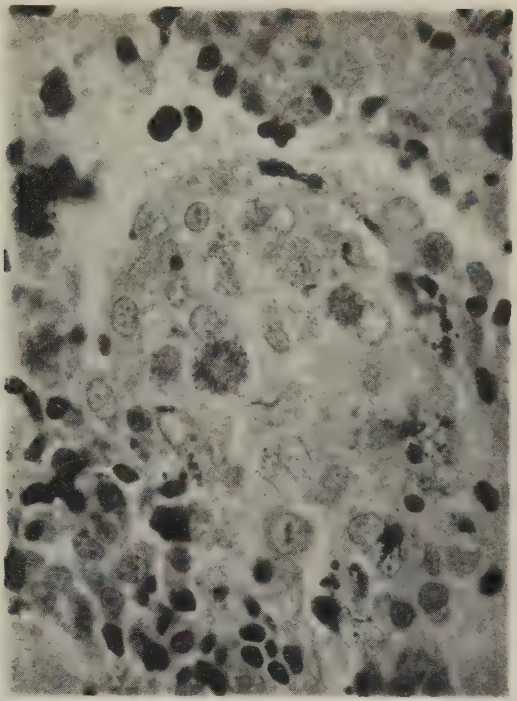

FIG. 1.

Young tubercle with characteristic rosettes of neutral red in epithelioid cells. The short, dark rods are tubercle bacilli stained with carbol fuchsin.

TABLE I. *Peripheral Blood.*

Date 1926	No. of Rabbits	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	Mono.
July 21/26	10	8,670	59.8	2.45	5.8	19.4	3.0	9.8
July 27	10	½ cc. Emulsion Bovine Tb. Bacilli—3 weeks culture—35 to Oil Imm. Field						
July 30 to Aug. 3	9	7,940	56.9	—	—	14.5	1.9	19.3
Aug. 4/11	8	10,500	56.2	0.5	4.6	15.3	3.3	20.5
Aug. 13/21	7	10,650	39.1	1.1	3.7	26.5	3.5	26.1
Aug. 28	3	8,030	40.0	1.3	4.3	24.3	2.3	28.3

Cunningham, Sabin, Sugiyama and Kindwall[2] have recently concluded, from studies of supravitally stained films taken from the cut surface of the lungs of tuberculous animals, that the epithelioid cell is a modified monocyte.

Though we have confirmed all of their observations by repetition of their experiments, and in sections of lung tissue have actually

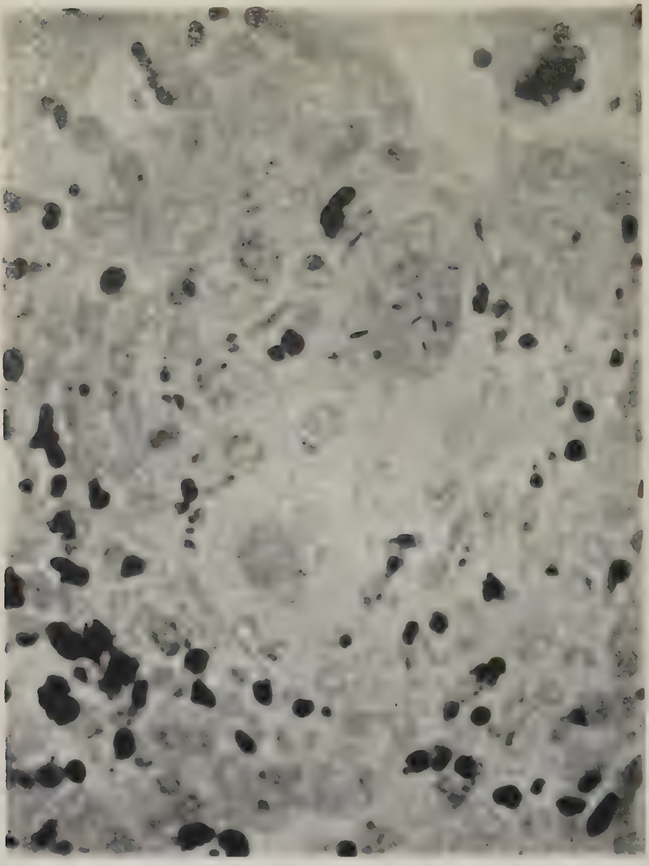

FIG. 2.

Slightly older tubercle showing two multinucleated epithelioid cells (giant cells). In each case the nuclei are arranged at the periphery of a single large neutral red rosette. These two cells contain many tubercle bacilli.

seen that the epithelioid cells are vitally stained with neutral red in the same manner as are the monocytes of the blood, we do not, at present, wish to support their interpretation of their findings, or take any other stand as to the origin of the epithelioid cell. The present report merely describes a method of study, and calls attention to certain striking vital staining characteristics of the epithelioid cells of tubercles, formed in the lungs, which have been demonstrated in sections.

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[1] Sabin, Florence R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 277.

[2] Cunningham, Sabin, Sugiyama and Kindwall, *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 231.

3292

Demonstration of a Gastric Secretory Excitant in Circulating Blood by Vivi-Dialysis.

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Since it has been proved that the transplanted stomach will secrete after a meal (Ivy and Farrel,[1] Lim, Loo and Liu[2]), it has become necessary to obtain the exciting substance or substances from the blood, as the first step towards identification. We have, therefore, attempted to obtain the excitant (or excitants) by dialysing the circulating blood of dogs during digestion.

The animals were either narcotised with amytal, or given no anesthetic at all. The vessels were exposed (under novocaine in the latter case), and connected with a set of dialysing membranes (Necheles[3]) immersed in warm Locke's solution. The blood was rendered incoagulable by recurring injections of heparin (Howell) or hirudin, or both. Circulation through the membranes was permitted for periods up to 5 hours, usually 2 hours, with the animal fed, 200 gr. meat and 100-200 cc. water, before or sometimes during the observation. The dialysate, concentrated or unconcentrated, was injected, subcutaneously, intravenously or intraperitoneally, into gastric pouch of dogs, whose secretory ability was determined by meal or histamine stimulation.

Three types of experiment were performed:

(1) Carotid artery and external jugular vein connected with membranes (carotid dialysis).

(2) Portal vein and external jugular vein connected with membranes (portal dialysis).

(3) Inferior *vena cava,* in thorax, and external jugular vein connected with membranes (caval dialysis).

The results in 14 satisfactory experiments were as follows:

Carotid dialysis: Six experiments, with no anesthetic except novocaine locally, gave 2 dialysates doubtfully positive, 4 negative. The experiments, under amytal, gave both dialysates feebly positive(?) after a long latent period, 2 and 4 hours.

Portal dialysis: Five experiments (under amytal), gave 2 dialysates undoubtedly positive, latent period within first hour, 2 probably positive and 1 negative.

Caval dialysis: One experiment, under amytal, gave dialysate positive.

These results prove that a gastric secretory excitant occurs in the blood, and that it is more readily recovered from the portal and inferior caval blood than from the systemic circulation, in which it is probably less concentrated. It is possible that the excitant may be found in the blood before meals. Note the one carotid dialysate obtained before feeding which gave a slightly positive result. These experiments suggest that the excitant is increased after meals.

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[1] Ivy, A. C., and Farrel, J. I., *Am. J. Physiol.*, 1925, lxxiv, 639.

[2] Lim, R. K. S., Loo, C. T., and Liu, A. C., *Chinese J. Physiol.*, 1927, i, (in press).

[3] Necheles, H., *Klin. Woch.*, 1923, ii, 27; *Chinese J. Physiol.*, 1927, i, (in press).

3293

Production of Hyperglycemia in Rabbits by Subcutaneous Injections of Magnesium Salts.

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The presence of magnesium in chlorophyll, and the important role of the latter in the metabolism of carbohydrates in plants, led us to study the effect of magnesium salts on blood sugar in rabbits. Five normal rabbits, starved for 24 hours, were injected subcutaneously with a 5 per cent solution of $MgCl_2$ or $MgSO_4$, using 5 cc. of this solution per kilo of body weight. During the experiment the rabbits were kept quiet in a tray, without restraint, and there was no struggle when the 2 cc. of blood were taken from the marginal ear vein. Such moderate bleeding does not affect essentially the blood sugar level in rabbits, as shown in a previous paper[1].

The results of the experiments are presented in the following table:

Rabbit No.	Magnesium salt solution (5 per cent)	Time of injection	Time when blood was taken	Glucose in blood	Hemoglobin (in per cent of normal human values)
				mg. per 100 cc.	
1	$MgCl_2$	9:40 a. m.	9:35 a. m.	121	
			11:40 a. m.	200	
2	$MgCl_2$	9:50 a. m.	9:45 a. m.	118	
			11:50 a. m.	250	
3	$MgCl_2$	9:35 a. m.	9:30 a. m.	125	
			10:00 a. m.	143	
			11:00 a. m.	182	
			12 noon	222	
4	$MgSO_4$	9:30 a. m.	9:25 a. m.	117	42
			10:00 a. m.	133	40
			11:00 a. m.	154	44
			12 noon	182	43
5	$MgSO_4$	10:00 a. m.	9:30 a. m.	125	
			12 noon	222	
			3:00 p. m.	143	
			9:30 a. m. (the following day)	105	

This table shows that subcutaneous injection of magnesium salts produces a marked hyperglycemia, with a maximum in from 2 to 2 1/2 hours. In 5 1/2 hours the blood sugar is not far from its initial level, and in 24 hours it has reached a normal value. Controls, on the hemoglobin content, show that the hyperglycemia is not due to a change in blood concentration. Similarity in the results obtained by subcutaneous injections of $MgCl_2$ and $MgSO_4$ is a sufficient proof that the hyperglycemic effect is due to the magnesium ion.

Our data throw light on the understanding of the therapeutic value of magnesium salts, given *per os* or intravenously, especially in the treatment of nephritic uremia and eclampsia. According to Blackfan[2], the treatment of uremia (occurring in the course of acute glomerular nephritis) by intravenous injections of 1 per cent magnesium sulphate, has given most favorable results. Lazard, Irwin and Vruwink[3], report that intravenous administration of magnesium sulphate in sufficient dosage will prevent the development of convulsions in eclampsia, and will control them after their onset. Titus, Hoffman and Givens[4], and also Titus and Givens[5], say that the intravenous injection of glucose is a valuable therapeutic measure in toxemia of pregnancy, and that, theoretically, there is no reason why it should not be of value in the various toxemias not related to pregnancy.

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[1] Horvath, A. A., *J. Biol. Chem.*, (in press).

[2] Blackfan, K. D., *Bull. Johns Hopkins Hosp.*, 1926, xxxix, 69.

[3] Lazard, E. M., Irwin, J. C., and Vruwink, J., *Am. J. Obst. and Gyn.*, 1926 xii, 104.

[4] Titus, P., Hoffmann, G., and Givens, M., *J. Am. Med. Assn.*, 1920, lxxiv, 777.

[5] Titus, P., and Givens, M., *J. Am. Med. Assn.*, 1922, lxxviii, 92.

3294

Trichomoniasis in Kittens.

JOHN F. KESSEL.

*From the Parasitology Laboratory, Department of Pathology, Peking Union Medical College.**

Da Cunha and Muniz[1] in South America have reported the finding in a cat of a flagellate to which they have given the name, *Trichomonas felis.* Brumpt[2] later records Trichomonas in two kittens in Paris. One of these kittens had been previously infected experimentally with human feces containing *Endamoeba dysenteriae,* and it therefore is possible that this Trichomonas had been derived from the human source. Brumpt did not state definitely whether opportunity of laboratory transmission was afforded the second kitten. He transferred these flagellates to other kittens, and accepted the name *Trichomonas felis* for them, instead of considering the possibility that the infections might have been derived from other animals, supporting his claim primarily on the basis of the negative transmission results of Hogue[3], who had been unsuccessful in establishing *Trichomonas hominis* from man in kittens.

During the spring and summer of 1926, while engaged in a study of experimental amoebiasis in kittens, the writer found 9 kittens, purchased from the streets of Peking, which showed natural infections of Trichomonas. This Trichomonas, as studied in saline and iodine-eosin smears, did not differ morphologically from the Trichomonas described by Brumpt, from kittens, nor from the common *Trichomonas hominis,* from man. The flagellar count of the majority of the specimens was four. The infected kittens showed a diarrhea consisting of a yellowish-brown stool, and in two instances a dysentery. These conditions were uncomplicated, for the most part, by other protozoan or pathogenic bacterial infections, and the kittens gradually wasted, and died within a week of the time when the infection was detected.

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* Contribution No. 83.

Upon culturing in Boeck's L. E. S. medium, the trichomonads were recovered and transferred experimentally to other tested negative kittens, two of which received the infection by mouth and four by anus. The infection was established in 5 of these kittens, 4 of which showed the same symptoms as the naturally infected ones, and died within 12 days.

In this experiment, and in the series recorded below, negative kittens were chosen in the following way: An enema was given, using a Luer syringe to which a small rubber catheter was attached. Fecal material, taken in this manner on three different days, was subjected to both microscopic examination, and to culture, before a kitten was assumed to be negative. Of 69 kittens thus tested and used in experimental amoebiasis work, only 3 developed a Trichomonas infection later. Of these three infections two were definitely traceable to a human source, while one was doubtful. Negative control cats remained healthy during the experiment.

Twenty-six tested negative kittens were experimentally infected with trichomonads from man, the monkey and the rat, with positive results in 10 out of 14 cases in which *Trichomonas hominis* was employed, in 2 out of 6 in which a four-flagellate Trichomonas from the monkey was utilized, and in 5 out of 6 in which *Trichomonas parva* from the white rat was present. The infection was transmitted both by mouth and by rectum, and diarrheic symptoms like the ones encountered in the naturally infected kittens were observed.

The pathological findings in the sections from the large intestine consisted in a catarrhal inflammation, with hypersecretion of the mucus membrane, together with a necrosis of the superficial layer, which in some instances had progressed to a superficial ulceration, Trichomonads were found in the necrotic layer, as well as between the cells of the mucosa, and within the lumen of enlarged glands.

From the observations here recorded the writer concludes that trichomonads from man, the monkey and the rat may be transferred to kittens, and that these infections, together with natural trichomonad infections in kittens, are associated with acute diarrhea in this host. The fact that trichomonads from these mammals, including man, have been established experimentally in kittens leads one to question whether *T. felis* is in reality a species peculiar to cats, or whether the trichomonads found in naturally infected kittens may not be the result of trichomonad infections derived naturally from other animals.

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[1] Da Cunha, A. M., et Muniz, J., *Brazil medico*, 1922, xxxvi, 285.

[2] Brumpt, E., *Ann. de Parasitol.*, 1922, iii, 239-251.

[3] Hogue, M. J., *Johns Hopkins Hosp. Bull.*, 1922, xxxiii, 427-440.

Iowa Branch.

State University of Iowa, Iowa City, Iowa, November 3, 1926.

3295

Coagulation Time Following Hemorrhage After Splenectomy.

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In 1914 H. Gray and L. K. Lunt[1] reported that blood obtained from a cat, which had been subjected to a hemorrhage amounting to 13 per cent of its blood volume, coagulated at a much greater rapidity than the blood obtained from the same animal prior to the hemorrhage. The most important organs having to do with this change were found by Gray and Lunt to be located below the diaphragm and they expressed the opinion that the liver, together with the intestines and possibly the adrenals, were probably responsible for the addition of substances which made the increased rapidity of coagulation possible.

In 1924 Barcroft, Harris, Orahovats and Weiss[2] reported upon the contraction of the spleen under conditions which require an increased amount of circulating hemoglobin. In hemorrhage, the spleen, by contraction, was found to be able in the cat to fully compensate for a loss of 1/15 of the total blood volume.

Our experiments were undertaken to determine what influence this ejection of splenic blood in hemorrhage might have in causing the increased rapidity of coagulation. Dogs were used in all the experiments and in some cases the coagulation time was obtained before and after hemorrhage both prior to splenectomy as well as afterwards. In the last few animals the tests were made only after splenectomy. Periods varying from 1 week to 3 1/2 months were allowed to elapse between the date of the splenectomy and the time of the hemorrhage experiment. Blood for each test was drawn, with a clean dry hypodermic needle, from exposed veins, and the coagulation time was determined in all cases by two methods, the

Dale and Laidlaw[3] and the Peterson and Mills[4]. The time recorded in seconds is from the moment the blood appeared in the syringe until the end point was reached. Great care was exerted to insure obtaining circulating blood for each sample, and to maintain the same technic in handling all samples of blood.

Typical results are shown in Tables I and II. After splenectomy the increased rapidity of coagulation did not occur, and our results indicate that the blood forced out by the splenic contraction, at the time of hemorrhage, contains elements which hasten the coagulating process.

TABLE I.
Dog B. Wgt. 5.2 kgm.
3/3/26 Normal Animal

Time	D. L* Seconds	P. M.† Seconds
10:39	135	105
10:43	135	105
10:47	165	105
10:57	Hemorrhage 39 cc.	
10:58	135	105
11:03	60	60
11:12	30	30
11:14	15-30	15-30
Splenectomy, March 22, 1926		
4/1/26		
10:48	145	120
10:52	135	120
10:55	120	105
11:06	Hemorrhage 55 cc.	
11:07	120	120
11:11	180	120
11:19	180	105
11:26	165	120
11:32	Hemorrhage 55 cc.	
11:37	135	110
11:42	150	135

* D. L. Dale-Laidlaw Method.
† P. M. Peterson-Mills Method.

TABLE II.
Dog L. Wgt. 10.8 kgm.
Splenectomy, July 9, 1926.
10/2/26

Time	D. L* Seconds	P. M.† Seconds
10:40	195	135
10:45	240	135
10:50	195	120
10:57	Hemorrhage 118 cc.	
10:58	195	135
11:02	165	120
11:07	195	120
11:12	225	120
11:17	Hemorrhage 117 cc.	
11:18	205	135
11:24	255	135
11:29	195	135
11:34	225	135

* D. L. Dale-Laidlaw Method.
† P. M. Peterson-Mills Method.

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[1] Gray, H., and Lunt, L. K., *Am. J. Physiol.*, 1914, xxxiv, 332.

[2] Barcroft, J., Harris, H. A., Orahovats, D., and Weiss, R., *J. Physiol.*, 1925, lx, 443.

[3] Dale, H. H., and Laidlaw, P. P., *J. Path. and Bact.*, 1912, xvi, 351.

[4] Peterson, M. F., and Mills, C. A., *Arch. Int. Med.*, 1923, xxxii, 188.

3296

The Effect upon Amphibian Differentiation of Feeding Iodofibrin, Iodoedestin and Iodogliadin.

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From the Zoological Laboratory, State University of Iowa.

Previous work by the writer[1] showed that the blood proteins, serum albumen and globulin, when fed to normal, thyroidectomized, or hypophysectomized frog larvae, exert no effect upon differentiation; but when properly iodized these same substances greatly accelerated the differentiation changes incident to metamorphosis. It will be recalled that thyroidectomized and hypophysectomized tadpoles do not metamorphose, but permanently retain their larval characters unless fed, injected, or transplanted with thyroid.

Iodofibrin.

Ten grams of pure fibrin from beef blood was pulverized and mixed with five grams of KI and two and a half grams of KIO_3. To this mixture was added a solution containing 2 cc. of concentrated H_2SO_4 and 300 cc. of water. The material was heated in a water bath at 90°—100° C. for 4 hours and stirred frequently. It was then filtered, washed first with water and then with dilute NH_4OH, followed by washing with water, alcohol and ether. The resulting product was iodofibrin.

Thirty completely hypophysectomized *Rana sylvatica* tadpoles averaging 30 mm. total length and showing no indications of metamorphosis were fed iodofibrin. Within 24 days, the entire culture, with the exception of 7 larvae which died at various stages of transformation, had differentiated to the stage where the animals had fore and hind limbs, frog mouth and marked tail resorption. All of the animals died at this stage of transformation.

A control culture of 30 hypophysectomized larvae fed non-iodized fibrin showed no metamorphic change at the end of 30 days feeding. The same experiment was repeated on completely thyroidectomized tadpoles with similar results.

Iodoedestin and Iodogliadin.

These 2 proteins were purchased in Germany and the method of iodization is unknown to the writer. Two series of 20 animals each, of thyroidectomized and hypophysectomized frog larvae, were fed iodoedestin and iodogliadin. Every third day a small quantity of algae were fed along with the iodized proteins. The hind limbs of the animals grew rapidly at first, but later, limb growth slowed down and metamorphic changes progressed slowly. It required 3 months of continuous feeding to bring the animals of these cultures to the same stage of metamorphosis as that which resulted from 20 days feeding with iodofibrin.

It was noted that considerable of the iodine split off from the edestin and gliadin after the powdered material had been in the water for an hour. Such was not the case with iodofibrin. It seems probable that the method of iodization employed has much to do with the activity of iodized proteins in accelerating amphibian transformation.

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[1] Swingle, W. W., *Biol. Bul.*, 1923, xlv, 229.

3297

The Effect of Forcing Fluids, upon Survival after Bilateral Epinephrectomy.

E. L. COREY. (Introduced by W. W. Swingle.)

From the Osborn Zoological Laboratory, Yale University.

Previous work done by several investigators has shown that the administration of certain solutions may result in prolongation of life beyond the usual period in epinephrectomized animals. Stewart and Rogoff[1], working on dogs, report that intravenous injections of Ringer's Solution and dextrose prolonged life. Zwemer[2], working on cats, in this laboratory, was able to keep double operated animals alive for considerable periods by administering orally 5 per cent solution of glucose. This work suggested the possibility that forcing fluids containing various substances might prove beneficial in the treatment of adrenal insufficiency. Zwemer and Swingle have noted and commented upon the symptoms of dehydration which appear after bilateral epinephrectomy in cats, and, the fact that animals, presenting marked symptoms, eagerly drink large quantities of water up to within a few hours of death.

The procedure followed in the present work was to remove the left adrenal and after a lapse of 5 to 10 days extirpate the remaining glands. Untreated cats subjected to this procedure generally survived about 60 hours. The solutions were given by stomach tube, beginning immediately after the second operation. None of the animals employed were pregnant.

Ringer's Solution definitely prolonged life, the average survival period of animals receiving it being 174 hours. Doubling the percentage of the constituents of Ringer's Solution did not materially prolong the life span.

Sodium chloride given in 1 per cent solution prolonged life, the average life span of animals so treated being 149 hours.

Sodium bicarbonate likewise increased the life span, the average being 143.3 hours for animals treated with it.

Sodium acetate did not increase the life span, the average being 60 hours. The ineffectiveness of the acetate was probably due to its irritating effect upon the alimentary tract since the cats had difficulty in retaining it.

Solution A_1 containing magnesium lactate 0.9, strontium lactate 0.024, acid sodium phosphate 0.024 prolonged the survival period to an average of 150 hours.

On the other hand, Solution A_2 containing magnesium sulphate 0.9, strontium chloride 0.024, sodium sulphate 0.024 failed to prolong life, the average survival being 68.5 hours.

A 5 per cent solution of glucose increased the life span to an average of 180 hours.

It is not clear why forcing fluids should prolong the life of bilaterally epinephrectomized cats. The idea which most readily comes to mind in this connection is that the forcing of fluids and the consequent diuresis flushes some toxic substance out of the organism. Such treatment is merely palliative since all animals so treated eventually succumb with typical adrenal symptoms.

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[1] Stewart, G. M., and Rogoff, G. M., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 394.

[2] Zwemer, R. L., *Anat. Rec.*, 1925, xxxi, 338.

3298

Blood Changes Following Bilateral Epinephrectomy in Cats.

W. W. SWINGLE.

From the Osborn Zoological Laboratory, Yale University.

During the last 3 years an intensive study of the functional significance of the suprarenal cortex has been carried on in this laboratory by the writer and his students. The present report is concerned with changes which occur in certain blood constituents following adrenal removal in cats.

It is well known that unilateral epinephrectomy is without demonstrable effect upon cats, hence animals subjected to such an operation were employed as controls. An interval of 5 to 7 days was allowed to lapse before the remaining adrenal was removed. Animals so treated generally survived loss of both adrenals for about 60 hours if untreated.

Phosphorus: The serum phosphorus shows a striking and consistent rise, following bilateral adrenal removal. The increase is considerable, especially when the animal is in the pre-coma or coma stage. Values as high as 15 to 16.5 mgm. per 100 cc. are not uncommon. The accumulation of phosphorus occurs simultaneously with the development of serious symptoms. Animals presenting slight symptoms do not show significant rises in phosphorus. The increase in serum phosphorus is apparently not due to increased blood concentration.

Calcium and *Sodium:* The results of repeated analyses are not of any significance. In general the calcium rises slightly and the sodium falls to about the same degree as that following bilateral epinephrectomy.

Non-protein nitrogen: The non-protein nitrogen always shows a sharp rise following adrenal removal, and occasionally the increase is very great. The average value for non-protein nitrogen, in unilateral operated cats, is between 42 to 47 mgm. per 100 cc., whereas, the average amount found in the blood of the double operated animals, presenting marked symptoms, is about 91 mgm. The non-protein nitrogen is highest when the animal is in or verging on coma. The increase becomes apparent within the first 24 hours after operation, and slowly increases, until the cat enters the precoma stage. Then a sharp rise occurs. Our highest figure for non-protein nitrogen was 170 mgm. per 100 cc.

Urea: The urea increases markedly after double operation, the normal value being 19 to 24 mgm.; whereas values ranging from 40 to 122 mgm. were observed in animals showing marked symptoms.

Protein and total nitrogen: These substances increase after the second operation. However, the increase in serum protein is not so great as we might expect, considering the apparent increase in blood concentration. The blood of animals in the pre-coma stage is concentrated, does not flow readily, clots rapidly, and yields small amounts of serum.

Blood Sugar: The blood sugar of normal cats ranges from 105 to 118 mgm. per 100 cc. Following bilateral operation the sugar drops markedly. The fall begins about 20 hours after the operation and continues slowly until death supervenes some 60 hours later. The blood sugar of cats verging on coma, 60 to 72 hours after operation, ranges from 60 to 40 mgm. per 100 cc. It was observed that the onset of symptoms of weakness and muscular asthenia in double operated cats coincides with the drop in blood sugar. Animals beginning to show muscular weakness and cerebellar symptoms, when walking, had blood sugar ranging from 80 to 75 mgm. As the sugar fell the weakness became more pronounced until finally, when coma developed, the blood sugar had fallen to 40 to 55 mgm. per 100 cc.

The pronounced hypoglycemia, following adrenal removal in cats, is not the cause of adrenal death, since animals injected with glucose eventually die with high blood sugar, although the life span of animals so treated is considerably prolonged. It is interesting in this connection to note that normal cats, injected with insulin, pass into coma and convulsions when the blood sugar reaches the same low level (40 to 55 mgm.) as that of bilaterally epinephrectomized cats in coma or convulsions. Our low figures for blood sugar were obtained from blood taken just before death.

The urine: The quantity of urine passed decreases sharply with the onset of serious symptoms. On the day when death occurs, or on the previous day, the animals void little urine. Some cats show almost complete suppression, others are able to pass small quantities until a few hours before death. Small amounts of albumin appear in the urine of animals showing marked symptoms.

Because of the phosphorus and nitrogen retention and the presence of albumin in the urine, the kidneys of double operated animals were studied histologically for degenerative changes. The results were disappointing in that, aside from hemorrhagic areas and congestion, clean cut pathological changes were absent. Nevertheless, the data indicate that the kidney is somewhat involved in the syndrome, which results in death following bilateral epinephrectomy. Experiments[1] on the acid-base equilibrium of epinephrectomized cats revealed the fact that one of the train of events leading to death from adrenal insufficiency is an uncompensated, non-volatile acidosis, which appears to be due to an increase of phosphoric and organic acids.

The complete data indicate that one of the causes of death following removal or destruction of the suprarenal cortex is acid intoxication due to accumulation and retention of the acid end products of normal metabolism. In the absence of the cortex the kidneys apparently fail to properly perform their acid eliminating function in relation to the neutrality regulation of the body. For some time, in some of our cases for 5 or 6 days, the respiratory mechanism is adequate to take care of the slowly advancing acid intoxication by throwing off volatile acid and the pH remains within the normal range. Finally there comes a time when the respiratory mechanism proves unequal to the task, and the acidosis becomes uncompensated, with falling pH and marked symptoms. The respiratory mechanism breaks down and death ensues from respiratory failure.

The type of acidosis, which develops following adrenal removal, is of a similar nature to the acidosis characteristic of the terminal stage of certain types of nephritis. It is suggested that the adrenal gland (cortex probably) supplies a hormone which is necessary for the maintenance of normal kidney function.

Complete papers presenting in detail the data summarized in this report will appear shortly in the American Journal of Physiology.

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[1] Swingle, W. W., and Eisenmann, A. J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 212.

3299

The Prevention of Tetany by the Oral Administration of Ammonium Chloride.

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From the Osborn Zoological Laboratory, Yale University.

Previous experiments[1, 2] carried out on parathyroidectomized dogs have demonstrated the effectiveness of the lactates of strontium and magnesium in relieving tetany and in bringing about a cure.

Experiments carried out last spring show that ammonium chloride also is an effective agent.

Parathyroidectomized dogs given a 5 per cent solution of ammonium chloride in 100 cc. doses twice daily, 3 to 4 hours before, and 5 to 8 hours after feeding, will retain the solution and may be kept free from tetany for long periods. One dog, reported here, never showed any symptoms of tetany save for a slight depression 4 days after operation. Once tetany appears, and the serum calcium falls below 7 mgm. per 100 cc., the ammonium chloride brings about a recovery within 2 hours, usually 1 hour and 15 minutes after administration. Examinations of the serum calcium at this time shows that the calcium has risen, on the average 2½ mgm. or to 8.5 mgm. per 100 cc., which is above the level at which tetany appears in dogs.

The experiments indicate clearly that ammonium chloride exerts a marked influence on tetany, probably more through its acidotic effect on the organism, than through an immediate effect on the serum calcium, although the rise in serum calcium may be sufficient to cause the disappearance of tetany symptoms.

Six dogs were parathyroidectomized. Dogs 4 and 6 were allowed to develop tetany; they recovered in 1 1/4 hours after receiving 100 cc. of 5 per cent solution NH_4Cl. The calcium of dog 6 rose from 6.6 mgm. to 10.1 mgm. per 100 cc. This was the greatest rise obtained for the 1 1/4-hour period. Dog 4 remained free from tetany for 4 days after the beginning of the ammonium chloride treatment, but died in tetany on the 6th day. Dog 6 recovered permanently. Animals 1, 2, 3 and 5 received ammonium chloride immediately after operation and twice daily thereafter. They showed marked tetany at different periods, followed by recovery after heavy doses of NH_4Cl. Dog 1 was depressed the 4th day after operation, but showed no other symptoms. Dogs 1, 2 and 5 recovered permanently. Dog 3 died of tetany and pneumonia on the 45th day.

After 30 to 40 days of ammonium chloride therapy the animals were placed on a meat diet and the ammonium chloride discontinued without ill-effects except in the case of animal 3.

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[1] Swingle, W. W., and Wenner, W. F., *Am. J. Physiol.*, lxxv, No. 2, 378-392.

[2] Wenner, W. F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 432-434.

3300

The Acid-base Equilibrium of Epinephrectomized Cats.

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Because of striking changes in certain of the blood constituents, especially phosphorus, which follow adrenal extirpation in cats, and the fact that these animals present unusual changes in respiration a few hours preceding coma and death, it seemed probable that acid intoxication was one of the train of symptoms leading to death from epinephrectomy.

Unilaterally operated animals were used as controls. The amount of serum protein, total nitrogen, non-protein nitrogen, serum carbon dioxide, serum chloride, inorganic phosphorus and total base was determined. The pH was also determined.

The second adrenal was extirpated from 5 to 7 days after the removal of the first gland. The blood determinations were made again when the animals showed marked symptoms of adrenal insufficiency.

The blood was obtained with the usual precautions, and all the determinations were made accroding to standard procedure. The acid values of protein, bicarbonate, chloride, and phosphorus were calculated as described by Peters *et al.*[1] Total acid was taken to be the sum of all the determined acids. Organic acid was calculated by subtracting total acid from total base.

The results of the blood determinations showed that marked changes occurred in the blood following bilateral adrenal removal.

1. The tension of carbon-dioxide always dropped.
2. There was always a fall in pH.
3. The proteins rose in all except one case.
4. There was usually a marked rise in phosphorus.
5. The serum bicarbonate always diminished.
6. The total acid always diminished (*i. e.,* at the expense of bicarbonate).

Certain values showed variation in different individuals.

1. Changes in chloride concentration were variable.
2. Total base showed a striking drop in three animals.
3. Organic acid was not consistently affected, although it tended to be higher in the operated animals.

The fall, in pH, carbon-dioxide tension, serum bicarbonate and total acid, indicates that the bilaterally operated animals showing marked symptoms of adrenal insufficiency were suffering from an uncompensated non-volatile acidosis. This seems to be due to an increase of phosphoric and organic acids. In the early stages of adrenal insufficiency the acidosis is of the compensated type.

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[1] Peters, J. B., Bulger, H. A., Eisenmann, A. J., and Lee, C., *J. Biol. Chem.*, 1926, lxvii, 141.

3301

The Influence of Pituitrin Administration upon Certain Phases of Carbohydrate Metabolism.

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Cushing[1] and coworkers have demonstrated that the injection of extracts of the posterior lobe of the pituitary gland results in a lowering of the tolerance for carbohydrates. The purpose of this investigation was to seek further information concerning the mechanism involved in this well known action of pituitrin.

We have made a comparison of the response to intravenous glucose administration in 6 dogs with and without the injection of pituitrin. Glucose was injected intravenously by means of a Woodyatt pump, for a period of 2 to 3 hours, at the rate of either 1.8 or 4 gms. per kilo body weight per hour. A continuous intravenous administration, of approximately .05 cc. of commercial pituitrin (Parke Davis and Co. or Eli Lilly and Co.) per kilo body

TABLE I.
Average Blood Sugar Values.

	4 gm. glucose per kg. hr.		1.8 gm. glucose per kg. hr.	
	Without Pituitrin	With Pituitrin	Without Pituitrin	With Pituitrin
Before injection	0.101	0.095	0.079	0.102
15 min. after injec. began	0.283	0.523		
30 min. after injec. began			0.231	0.315
1 hr. after injec. began	0.492	0.673	0.269	0.350
2 hrs. after injec. began	0.400	0.885	0.239	0.330
15 min. after injec. ended	0.218	0.472		
30 min. after injec. ended.			0.076	0.134

weight per hour, was made by means of a device similar to that described by Burn and Dale[2]. The method employed for studying the response was the same as previously described.[3]

Each animal receiving pituitrin showed a greater hyperglycemia (Table I) and a greater glycosuria than was observed in control experiments on the same animal. This effect was noted early in the course of the experiments, and was not more marked at the end of the period. The animals, receiving glucose at the rate of 4 grams per kilo per hour, excreted 18.1 per cent of this amount in the urine, as compared to 36.5 per cent for the same animals with glucose and pituitrin. When the injection rate was 1.8 gms. the per cent excreted was 10.7 and 25.6, respectively. The concentration of glucose in the urine was unaffected by pituitrin. Studies, made on the plasma pH and CO_2 content, indicated that pituitrin injection caused no appreciable change in the acid base balance of the blood.

TABLE II.

Average R. Q. and Calories per sq. meter Body Surface Area.

	R. Q.		Calories	
	Without Pituitrin	With Pituitrin	Without Pituitrin	With Pituitrin
Before injection	0.79	0.81	*40.7	*35.7
1 hr. after injec. began	0.99	1.00	59.5	60.2
2 hrs. after injec. began	0.96	0.89	58.5	62.7
3-5 hrs. after injec. began	0.83	0.87	39.2	44.8

* Because of the conditions of the experiments these values are to be considered as preliminary rather than basal.

Respiratory data (Table II) indicate that pituitrin has been without influence on the quantity or quality of the oxidative processes during glucose administration. The glucose injection was accompanied by a slight elevation of body temperature, a slight increase in pulse rate, and no change in respiration. The experiments with glucose and pituitrin administration show either no change or a slight fall of body temperature, a slowing of the pulse and somewhat accelerated respiration.

These results indicate that pituitrin has decreased the rate of removal of glucose from the blood by the tissues and that this has not been caused by acid base changes in the blood, or influenced by an altered kidney function. The quantity of glucose disposed of by oxidation was unaltered. The finding of a decreased pulse rate suggests that circulatory changes may be an important factor. Our results are, however, not opposed to an antagonistic action between insulin and pituitrin.[4]

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[1] Goetsch, E., Cushing, H., and Jacobson, C., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 165.

[2] Burn, J. H., and Dale, H. H., *J. Physiol.*, 1924, lix, 164.

[3] Boyd, J. D., Hines, H. M., and Leese, C. E., *Am. J. Physiol.*, 1925, lxxiv, 656.

[4] Burn, J. H., *J. Physiol.*, 1923, lvii, 318.

3302

Placental Transmission. IV: The Protein Fractions in Fetal and Maternal Plasma.

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The total protein and its albumin, globulin, and fibrin fractions were determined by the method of Wu in the plasmas from 15 mothers and infants, the blood specimens having been obtained at the time of birth.

The total protein in the fetal plasma was uniformly lower than in the maternal plasma, the average difference being 1.00 gram per cent. In maternal plasma, the albumin content was higher than in the fetal specimen in all but two instances. Serum globulin was always higher in the mother. The fibrin content of the maternal plasma was uniformly greater than that of the fetal plasma, the average figure for the former being 0.44 gram per cent and that for the latter 0.27 gram per cent. The plasma of the parturient woman contains less total protein and less albumin than does that of non-pregnant women, whereas the globulin is approximately the same and the fibrin is considerably increased during the latter part of pregnancy. The albumin, globulin, and fibrin percentages of total plasma proteins were strikingly constant in the various bloods, irrespective of the differences in their total protein content.

It was suggested that perhaps the excess of protein in the maternal blood compensates for the higher concentration of certain crystalloid substances in the fetal plasma, so that equal osmotic pressures may obtain on the two sides of the placental barrier.

The low fibrin values in fetal plasma may be related to its diminished viscosity, and to its lowered surface tension, as well as to the slower clotting time of fetal blood and to the increased stability of the fetal red cells.

Missouri Branch.

Washington University School of Medicine, November 3, 1926.

3303

The Effect of Light upon the Follicular Hormone.

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In an earlier paper we[1] described an improved method for the preparation of the oestrous hormone from hog liquor folliculi, which was based upon the differences in the solubilities of the hormone and cholesterol in petroleum ether and 70 per cent alcohol. During the past summer we were astonished to find that the procedure was not as satisfactory as was formerly found and that losses of over 50 per cent of the hormone were frequently encountered. Since the only changes in the procedure were those imposed by climatic conditions and the substitution of more carefully purified solvents for the commercial products, it was a relatively easy matter to determine the source of our trouble. By the process of elimination it was found that light exerts a profound effect upon the hormone. It is possible that this was not noticed earlier because the laboratory work upon which the method is based was carried out during the winter months.

In an earlier publication Allen and Ellis[2] call attention to the destruction of the hormone by ultra violet light. Some data, which confirm and extend theirs, have been obtained, but our most striking observations are upon the effect of diffuse daylight from north windows upon the activity of the hormone. That such inert (chemically) substances as hexane will cause a 50 per cent loss of hormone in two days, whereas preparations may be kept in ether or alcohol for two years with little loss, seems remarkable. The data of Tables I and II illustrate the effects of light upon the hormone under various conditions.

TABLE I.

No.	Volume Injected.						Remarks.
	.5 cc.	.6 cc.	.7 cc.	.8 cc.	.9 cc.	1.2 cc.	
24a	+						Stock solution—1st ether extraction.
24e		+					Quartz tube in ether 2 days diffuse light.
241		—		—		+	Ether soln. of hormone + alc. soln. of eosin in pyrex tube in diffuse light 2 days.
2411		+				+	Same as 241 except fluorescein was used.
24i		—			—	+	Ether soln. + ether soln. hemin in quartz tube in sunlight 1 hr.
24k		+				+	Same as 24i except alc. soln. hematoporphyrin used instead of hemin.
24h		±		++ (0.75 cc.)			26 hrs. in pet. ether in amber bottle.
24d		—				—	Quartz tube in pet. ether 2 days diffuse light.
24m		+				+	Two days in hexane (syn.) in amber bottle in diffuse light.
241		—			—	+	Same as 24m but kept in pyrex tube.
243m					±	+	Same but in quartz tube.
240		—	—		++		3 cc. ether soln. quartz tube in bright sunlight for 3 hrs.
241o		+					Same as 240 but dissolved in 1 cc. alcohol.
23d		+					Alc. soln. in sunlight for 1 hour.
23c		—				—	Same as 23d but containing trace of eosin.
23e		—				±	Ether-petroleum ether soln. exposed to sunlight for 20 min.
23f		—				—	Alc. soln. + crystal of quinine in sunlight for 45 min.

Symbols:
— less than 1 rat unit in volume injected.
+ one or more rat units in volume injected.
± almost 1 rat unit in volume injected.
++ prolonged period of positive reaction.

TABLE II.

No.	Units per 100 cc.		Date.		Remarks.
	1st assay	2nd assay	1st assay	2nd assay	All exposed in light from north windows.
159	66	57	Feb., 1924	Aug., 1926	Ether solution of liquor folliculi extract, green glass bottle.
6A32	333	200	May, 1925	Aug., 1926	Ether solution of placental and ovarian extract in clear flint glass.
R.R.	>500	<100	May, 1925	Aug., 1926	Petroleum ether solution of placental extract in amber bottle part(?) of time.
125	116	80	Dec., 1923	Aug., 1926	Alcoholic solution of a liquor folliculi extract in clear flint glass.

The method of preparation already referred to, but carried out by the illumination from a photographer's red light, gives almost total recovery of the hormone in a purified condition (0.01 to 0.02 mg. per rat unit).

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[1] Ralls, J. O., Jordan, C. N., and Doisy, E. A., *J. Biol. Chem.*, 1926, lxix, 357.

[2] Allen, E., and Ellis, M. M., *J. Am. Med. Assn.*, 1925, lxxxv, 94.

3304

Specific Effect of Salts in Extraction of Urease from Amoebocyte Tissue of Limulus.

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In a former communication[1] we have shown the presence of urease in the amoebocyte tissue, blood serum, muscle tissue and even in the unfertilized eggs of Limulus. In experiments carried out during the past summer we investigated means of extraction of this enzyme from experimental amoebocyte tissue, and we observed very striking differences in the extracting power of different salts.

Distilled water with or without the addition of urea is entirely or almost entirely ineffective. The Woods Hole sea water, which is alkaline, or sea water previously neutralized, heated blood serum of Limulus, or, serum from which the proteins have been removed through heating, show a very moderate degree of efficiency. Solutions of NaCl or KCl, approximately isotonic with sea water, behave in a similar manner to sea water, or are even slightly less effective than the latter. On the other hand, extracts prepared with isotonic solution of $CaCl_2$, $BaCl_2$ or $SrCl_2$ have a very high degree of activity. Solutions of $MgCl_2$ and also of $MnCl_2$ have an intermediate position, but approach more closely the former solutions than they do $CaCl_2$, $SrCl_2$ or $BaCl_2$. Solutions of $CuCl_2$ or $ZnCl_2$ are entirely or almost entirely without effect. Extracts made with isotonic solutions of $CaCl_2$, $BaCl_2$ or $SrCl_2$ are perhaps 40 to 100 times more active than extracts made with M/2 NaCl or M/2 KCl.

The anions tested thus far did not seem to possess a much greater extracting power than the corresponding chlorides. However, through increase in the concentration of various salts, we can increase their effectiveness. Thus the optimal concentration of NaCl seems to lie between 1.25 and 2.5 m, while in the case of $CaCl_2$ and also of $MgCl_2$ it seems to be approximately between 0.38 and 0.75 m. However, further investigations in this respect are needed. If, instead of comparing the effectiveness of isotonic solutions of various salts, we compare that of solutions representing the optimal concentrations of various salts, the advantage of $CaCl_2$, $BaCl_2$, and $SrCl_2$ over NaCl, although less pronounced, is still quite marked, the former being about 8 times more effective than the latter.

If we add $CaCl_2$ merely to the finished extracts made with solutions of NaCl, no noticeable improvement in the activity is observed. It is therefore the process of extraction as such which is improved through $CaCl_2$ and related salts rather than the action of the enzyme on urea.

Extracts made with $CaCl_2$, $SrCl_2$ or $BaCl_2$ differ still in another respect from all other extracts so far tested: in mixtures of urea solutions and extract they prevent the rise of the pH from 7.0 to 9.0, which normally takes place as the result of the conversion of urea into ammonium carbonate. This neutralizing power of $CaCl_2$ extracts seems to run parallel to their concentration in urease. It is therefore possible that it is a substance (protein?) specifically extracted by $CaCl_2$ and perhaps associated with the enzyme which is responsible for this neutralizing power. On the other hand, it can be shown that the neutralization as such is not the cause of the great effectiveness of $CaCl_2$ and similar salt solutions in the preparation of the urease. Variations in the pH of NaCl solutions may increase or diminish their effectiveness as extracting agents of urease, but the greatest activity thus attained remains considerably below that attainable through the use of salts with more favorable kations.

Our experiments prove thus that urease is extracted from amoebocyte tissue in a quantitatively graded way by certain kations and that among these Ca, Ba and Sr are by far the most effective.

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[1] Loeb, Leo, and Bodansky, Oscar, *J. Biol. Chem.*, 1925, lxvii, 79.

3305

The Presence of a Polysaccharide-Like Substance in Blood.

MICHAEL SOMOGYI AND ETHEL RONZONI. (Introduced by P. A. Shaffer.)

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In 1909 and the following years Lépine and Boulud[1] asserted that besides the usual form of sugar, blood contains a latent form of glucose which does not reduce alkaline copper solutions, but after being set free by acid hydrolysis behaves as ordinary glucose, reduces copper, and is fermented by yeast. This "sucre virtuel" plays an important part in carbohydrate metabolism. They believed that glucose and "sucre virtuel" were readily converted into one another, and that free sugar before utilization had to be incorporated into the larger complex molecule. In the diabetic this elastic shift from one form to the other was impaired. Sugar disappearing during glycolysis they claim to have recovered as "sucre virtuel." Lépine characterizes this sugar as glucose in combination with blood proteins in a glucoside linkage.

Lêpine's analytical method consisted of precipitation of the blood proteins by coagulation with Na_2SO_4 and heat. The coagulum was separated by filtration, washed and hydrolyzed by means of hydrofluoric acid in lead crucibles for from 22 to 30 hours. Each determination required 10 cc. portions of blood and the life of a rabbit. Few have tried to repeat Lépine's work, and those who have failed to confirm his observations and his theories have been entirely discredited.

During the early part of last year we observed that the blood of animals in extreme insulin hypoglycemia yielded, on heating in a water bath for 2 hours with N/2 HCl, a substance giving typical reduction of alkaline copper solutions, and which disappeared completely if incubated 24 hours with yeast.

This observation agreed so completely with those of Lépine that we believed we were dealing with the same substance. After working out a simple technique for its determination (consisting in hydrolysis, for 2 hours, with N/2 HCl, in a boiling water bath, with subsequent precipitation of protein by $Hg(NO_3)_2$), we found this sugar present in the blood of all animals examined: man, ox, sheep, dog, rabbit, and guinea pig, in varying concentration in different species and in different members of the same species. Further, investigation into its relation to carbohydrate metabolism was undertaken, and observations were made of its behaviour during changes produced in the blood sugar by the following procedures: (1) Ingestion of glucose, (2) injection of insulin, (3) glycolysis *in vitro,* (4) fermentation.

During sugar tolerance tests the latent sugar remains fairly constant and uninfluenced by the concentration of the blood sugar. Occasionally, there is contrary to Lépine's findings a slight fall when the glucose of the blood is at its highest.

The injection of insulin left the latent sugar near the usual level, in guinea pigs, in insulin convulsions. An experiment on rabbits also shows the failure of insulin to influence the level of the latent sugar. In one experiment in a diabetic patient, entering the hospital in coma, treatment with insulin caused a drop in blood sugar from 360 mgs. to 59 mgs., at which point there were symptoms of insulin reaction. The latent sugar showed relatively slight changes. It is evident that changes in combined sugar are not related to changes in ordinary blood glucose.

Our test tube experiments show quite conclusively that there is no change in the latent sugar during glycolysis of a duration long enough to remove completely the glucose from the blood. In fact, if the blood remains sterile, incubation for a period of 18 hours failed to influence the latent sugar.

Fermentation tests carefully controlled showed that our earlier observations when 24 hours incubation was used were worthless. In non-sterile blood, frequently without the addition of yeast, all reducing substances, including non-sugar, disappears during 24 hours incubation. With yeast, 3 to 4 hours incubation at 37.5° C is sufficient to remove the glucose from the blood, together with added glucose, or pure glucose from its solution. When hydrolized blood is fermented under these conditions the liberated latent sugar remains untouched. If the same sample of hydrolized blood is allowed to stand over night one finds but slight reducing properties remaining.

The non-fermentability of our reducing substance gave rise to the question of whether or not we were actually dealing with a sugar. The fact that the copper reduction is typical cannot decide this. We obtained typical osazone crystals, but only in sufficient quantity for an approximate determination of the melting point, which was about 180°C. The small amounts of latent sugar, which we were able to obtain free from glucose, we found to be dextrorotatory after hydrolysis, the rotation in relation to its reducing power being considerably less than that of glucose.

Because of the fact that this sugar is not contained in the protein free filtrate, regardless of the method of removing protein, we concluded that it is chemically combined with blood proteins. It remained to decide whether it was the monosaccharide of Lépine in glucoside linkage with the protein or Pavy's[2] amylose carbohydrate described in 1893. Following Pavy's technique we submitted the blood proteins to alkaline hydrolysis, as for glycogen determinations, subsequently precipitating with 10 volumes of alcohol. The precipitate was then separated and hydrolyzed by acid, giving a reducing substance qualitatively and quantitatively identical with that found in the same blood by direct acid hydrolysis. This shows that the combined carbohydrate of the blood is a polysaccharide.

Since this combined carbohydrate is largely contained in the plasma, we determined its distribution between globulin and albumin fractions. About 70 per cent is in the globulin fraction, the remainder being recovered from the albumin.

We have then, in blood, a substance combined with the protein which can withstand alkaline hydrolysis, which is susceptible to acid hydrolysis, as a result of which it yields a substance which gives a typical sugar reduction of alkaline copper solutions, which forms an osazone, which is dextrorotatory, with a rotation less than that of glucose, if based on its reduction value. It is not fermented by yeast after hydrolysis, nor is it susceptible to glycolysis. Its concentration varies in different individuals of the same species, and its level is independent of the amount of glucose in the blood and is unaffected by insulin injection.

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[1] Lépine, R., and Boulud, *J. de Physiol. et de Pathol. Général*, 1909, xi, 557; 1911, xiii, 178. *Compt. R. Soc. Biol.*, 1912, lxxiii, 589; 1913, lxxiv, 76.

[2] Pavy, F. W., "The Physiology of the Carbohydrates," J. & A. Churchill, 1894, London.

3306

The Starvation Ketosis of a Monkey.

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In sharp contrast to human beings, most animals (dog, cat, pig, steer, goat, guinea pig, rat, rabbit, etc.) appear to develop only slight ketosis when starved. In man, on the other hand, starvation ketosis is a regular phenomenon, and is apparently a necessary consequence of the failure to metabolize a sufficient amount of carbohydrate. And conversely it is believed that the metabolism of carbohydrate is essential for the normal complete combustion of the precursors of the acetone bodies. This seems applicable only to man. The ability to avoid ketosis without the direct intervention of metabolizing glucose appears to be possessed by most animals. Man is the exception. That animals have high tolerance for the acetone bodies is indicated by the results of acetoacetic acid injection (dog[1], mouse[2], cat[3]). This ability of animals to burn acetone bodies (apparently without the antiketogenic action of carbohydrate) would seem to be not an acquired property, but rather a function normal to all animals with the exception of man in whom it appears to have been lost. A parallel case is perhaps the ability to further metabolize uric acid to allantoin, possessed in general by mammalia, but lacked by man and the chimpanzee.

The following observation has been made on a fasting monkey, which developed a ketosis quite comparable to that of man which was promptly abolished by glucose and food. Whether the results are representative of the behavior of the species or of primates generally can be determined only after observations on other individuals, though the writer sees no reason to suppose that the behavior of this monkey is exceptional.

The subject was an adult male brown capuchin monkey, whose probable normal weight was 1.3 to 1.4 kilos. He was sent to the laboratory suffering with "cage paralysis."[4]

The animal was placed in a small metabolism cage in a room kept at a temperature of about 30°C. The animal always sat at one end of the cage and, except for motions of the head, was very quiet. The absence of activity permits an approximate estimate of the total metabolism. The starvation was continued through the third day during which urine was collected in short periods without catheterization.

At the end of three days glucose and food was given. *The ketosis disappeared almost immediately.* An increase in weight from 830 grams to 1,020 grams occurred in less than 24 hours after consumption of food.

That the ketosis is comparable to that of man is shown in Table I. When compared on the basis of body weight the ketosis of the monkey per kilo is more than three times as great as that of man. A more logical basis of comparison is on the basis of surface area

TABLE 1.

Comparison of Maximum Fasting Ketosis of Man and Monkey.

	Normal weight kilos	Estimated Total Metabolism* calories	Total Acetone Bodies Excreted		
			In 24 hours Total mMols.	Per kilo body wgt. mMols.	Per 100 Cal. heat output mMols.
Old-world monkey(?) (Baer)†	6.65		4.6	.7	
Capuchin monkey (Friedemann)	1.4	160	14.7	10.5	9.2
Subject "L" (Benedict)	61	1260	101	1.7	8.0
Fasting woman "M. K.", catatonic stupor (Grafe)	52	1325	167	3.2	12.6
Fasting obese woman (Means, Folin and Denis)		2039 1st fast	237		11.6
		1796 2nd fast	166		9.2
		1759 3rd fast	193		11.0

*Some of the estimates are cited by Shaffer, *J. Biol. Chem.*, 1922, liv, 399.

†Baer, J., *Arch. Exp. Path. u. Pharm.*, 1906, liv, 153.

or of total metabolism. The surface area was determined after death by two methods which agreed within 6 per cent. Measurements on various parts of the body and calculation of the surface area yielded a value of .143 sq. meter. The skin when stretched out on paper covered an area of .152 sq. meter. The calculations of the total metabolism were based on the following assumptions:

Surface area	.152	sq. meter
Basal metabolism per sq. meter	40	Cal.
Allowance for activity	10	per cent

This gives 160 Cal. per 24 hours. As is shown by the last column of Table I, the acetone body excretion per 100 Cal. heat output is of the same order of magnitude as that of man.

It is of interest to attempt an evaluation of the acetone body excretion on the basis of the ketogenic versus anti-ketogenic factors used by Shaffer.[5] The results of such calculations are shown in Table II. The expected or estimated excretion agrees remarkably

TABLE II.
Protocol of Experiment.

Period of Urine Collection.	Calculated to 24 hours.						
	Urine volume cc.	Aceto-acetic acid + acetone mMols.	B-oxy-butyric acid mMols	B-*oxy*:acetone. etc. Ratio	Total urinary nitrogen grams	Calculated total acetone bodies (Shaffer) mMols. in 24 hrs	Total acetone bodies excreted. mMols. in 24 hrs
Food eaten on Friday morning. Was brought to the laboratory Saturday and immediately placed in small metabolism cage.							
Saturday 12 M. to 5:30 P. M.	148	.07	.10	1.43	.742		
Sat. Sun. 5:30 P. M. to 7:30 A. M.	45	2.01	2.83	1.41	.308*		
Sun. Sun. 7:30 A. M. to 6:00 P. M.	325	5.46	6.05	1.11	.849	13.5	11.5
Sun. Mon. 6:00 P. M. to 9:30 A. M.	101	4.18	7.02	1.68	.785*	15.6	11.2
Mon. Mon. 9:30 A. M. to 2.30 P. M.	226	5.49	9.18	1.67	.820	15.0	14.7
At 2:30 P. M. drank about 25 cc. of 50 per cent glucose but vomited after 5 minutes. Urine specimen containing glucose was discarded at 5:00 P. M.							
At 5:00 P. M. injected 7-8 cc. of 25 per cent glucose subcutaneously. Immediately ate one-fourth of banana. During the night ate 2 bananas and about 100 cc. milk.							
Mon. Tues. 5:00 P. M. to 9:00 A. M.	30	.04	.16	4.00	.291		

*Vomited. Total nitrogen determination was made on $CuSO_4$ and $Ca(OH)_2$ filtrate, which was also used for B-oxybutyric acid determination.

well with the values found by analysis. This close agreement is consistent with the view that the marked ketosis in this animal was the consequence of the same factors which determine human ketosis.

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[1] Wilder, R. M., *J. Biol. Chem.*, 1917, xxxi, 59. Friedemann, T. E., Somogyi, M., and Webb, P. K., *J. Biol. Chem.*, 1926, lxvii. (Proc.) 44.

[2] Robertson, T. B., *Med. J. Australia*, 1923, ii, 191.

[3] Burn, J. H., *J. Physiol.*, 1925, lx, 16.

[4] Fox, H., ''Disease in Captive Wild Mammals and Birds.'' Lippincott, 1923.

[5] Shaffer, P. A., *J. Biol. Chem.*, 1922, liv, 399.

New York Meeting.

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3307

The Effect of Selection on the Rate of Reproduction of Paramecium Aurelia.

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The work of Jennings[1] affords much evidence that, in *Paramecium,* morphological differentiations, which are hereditary, do not arise in a line reproducing vegetatively. As a result, selection is powerless to isolate hereditarily different strains among the descendants of a single cell. On the other hand, the same author[2] has shown that in the uniparental reproduction of a rhizopod, *Difflugia corona,* the number and length of the spines on the test, as well as other morphological characters, become differentiated in the descendants of a single individual; and that by selection it is possible to isolate many hereditarily different strains.

The effectiveness of selection in regard to what may be termed a physiological character has not been tested in *Paramecium,* but Middleton[3] has undertaken this in a study of the fission rate of another ciliate, *Stylonychia pustulata.* This author found that "by opposite selection, through more than 150 generations, of small individual variations occurring among the progeny of a single individual, it was possible to produce two sets differing hereditarily in rate of fission." The hereditary difference persisted through periods of balanced selection, and through conjugation, and disappeared only under the influence of reversed selection.

In view of the above results a series of experiments were carried on which involved the selection of a physiological character in *Paramecium.*

Although there is a remarkable synchronism of fission in closely related cells of a race of *P. aurelia,* under identical conditions, frequently one cell will divide slightly earlier than the others. This variation in the rate of division of individual cells is the physiological character which was selected in our experiments.

TABLE I.

A—Opposite Selection—200 days.
'Fast' lines 262 generations.
'Slow' lines 196 generations.
Difference in favor of fast, 66 ± 1.20.

A Opposite Selection		A_1 Balanced Selection		A_2 Reversed Selection	
225 days		225 days		225 days	
'F' lines	298	'F'-Bal. lines	295	'F'-Rev. lines	295
'S' lines	220	'S'-Bal. lines	219	'S'-Rev. lines	223
Difference	78 ±1.75	Difference	76 ±1.15	Difference	72 ±1.23
250 days		250 days		250 days	
'F' lines	336	'F'-Bal. lines	326	'F'-Rev. lines	327
'S' lines	236	'S'-Bal. lines	237	'S'-Rev. lines	244
Difference	100 ±1.70	Difference	89 ±1.89	Difference	83 ±2.94
275 days		275 days		275 days	
'F' lines	371	'F'-Bal. lines	361	'F'-Rev. lines	348
'S' lines	258	'S'-Bal. lines	261	'S'-Rev. lines	267
Difference	113 ±2.11	Difference	100 ±2.77	Difference	81 ±4.62
300 days		300 days		300 days	
'F' lines	401	'F'-Bal. lines	391	'F'-Rev. lines	375
'S' lines	275	'S'-Bal. lines	272	'S'-Rev. lines	286
Difference	126 ±3.27	Difference	119 ±2.16	Difference	89 ±3.84

The experiments summarized in Table I extended over a period of 300 days. From the progeny of a single animal eight lines were isolated; four of these lines were subjected to what might be referred to as a fast, or plus, selection over this period, the other four to slow, or minus, selection. At the end of 200 days the two sets showed a marked diversity in the rate of fission. The fast set attained an average of 262 generations, the slow set 196 generations. As indicated in the table, there was a very significant difference of 66±1.20 generations between the fast and slow lines at this time.

At the 200-day point two sets of sister animals were isolated from each of the A lines. One set of eight lines (A_1) was subjected to balanced selection (which amounts to no selection) for 100 days. That is, each plus selection was followed by a minus selection, at the next opportunity, and *vice versa.* Another set of eight lines (A_2) was isolated from the main diverse lines of A, and upon this was practiced reversed selection. That is, the fast lines were now subjected to slow or minus selection, the slow lines to fast or plus selection.

During this period of 100 days opposite selection was continued in the main (A) lines until we have a difference of 126±3.27 generations between the fast and the slow lines. Throughout this same period the lines subjected to balanced selection (A_1) maintained a difference in division rate of 119±2.16 generations, while the difference in the case of the reversed lines (A_2) had dropped to 89±3.84. Thus (taking into consideration the probable error) we find that this difference, which was cumulative, has persisted through the period of balanced selection, but tends to disappear under the influence of reversed selection. Selection has apparently been effective in establishing two diverse sets, differing in regard to the physiological character, division rate.

A more detailed analysis of these experiments will be presented in another paper. The effect of selection is now being studied in *Paramecium calkinsi,* a form which differs from *Paramecium aurelia,* in that endomixis has never been demonstrated, and apparently does not occur. The experiments are also being repeated on the hypotrich, *Stylonychia pustulata,* for the purpose of comparison with the two holotrichous forms mentioned above.

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[1] Jennings, H. S., *Proc. Am. Phil. Soc.*, 1908, xlvii, 393.

[2] Jennings, H. S., *Genetics,* 1916, i, 407.

[3] Middleton, A. R., *J. Exp. Zool.*, 1915, xix, 451.

3308

Diabetes Mellitus: An Experimental Study on the Etiology of the Disease.

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A survey of the literature gives no convincing evidence that the disease is caused by bacteria. It seemed possible that it may be caused by a filterable virus.

Experiments were made on rabbits to determine whether it is possible to obtain evidence of the presence and action of a filterable virus. It seemed probable that the causative organism is thrown off from the patient's body in the urine. The urine of diabetic patients was therefore passed through Berkefeld filters, and the filtered urine was injected into the circulation of rabbits in doses of

2 cc. Two rabbits were injected with the urine of each patient, each animal receiving only one injection.

After the rabbits had been injected they were confined in metabolism cages which permitted the collection of the urine. Daily analysis of urine was carried out, using Benedict's quantitative reagent.

Diabetes mellitus is generally a slowly progressing disease and it was believed that if it is transmissible to rabbits evidences of infection would probably be delayed for some time.

Experiment 1. The first pair of rabbits was injected with the filtered urine of patient Q on January 12, 1926. Almost daily urine examination failed to show the presence of glucose until 22 days later, when a trace of sugar was found in the urine of rabbit No. 1 (female). Rabbit No. 2 (male) first showed glucose in the urine on the 42nd day after inoculation.

A peculiar phenomenon in the experimental glycosuria in rabbits has been the irregular periodicity of the occurrence of glucose in the urine. After glucose was demonstrated for several successive days it disappeared, but reappeared more or less irregularly. After glucose had been demonstrated for several months in the urine of these 2 rabbits they were taken out of the metabolism cages. Rabbit No. 2 was killed 152 days after inoculation, and Rabbit No. 1 was kept over the summer, and again placed in a metabolism cage 250 days after inoculation. Glucose was not found again in the urine of this rabbit until 279 days after inoculation, when it was present to the amount of 1.426 per cent, and continued to be voided in varying amounts for several days when it was again absent. The glucose in this specimen permitted active fermentation with gas formation by Fleischman's yeast at 37°C.

Experiment 2. The filtered (Berkefeld) urine of the patient (Q), when inoculated into serum-broth medium and grown in the incubator under aerobic conditions, appears to permit development of the causative agent, though no organisms could be demonstrated, and there was no visible change in the medium. After cultivation for 56 days this culture was inoculated into the circulation of normal rabbits in 0.1 cc. amounts. Glucose appeared in the urine in a shorter time (in 7 to 8 days, rabbits Nos. 5 and 6) than in those inoculated with the filtered urine of the patient, indicating that the culture contained many more organisms than the diabetic urine of the patient.

Experiment 3. Two rabbits were inoculated with a later culture, No. 8 with heated culture and No. 9 with unheated culture, but these failed to develop glycosuria in 28 days, when they were killed.

Experiment 4. The urine of rabbit No. 5 (inoculated with a serum broth culture of the filtered urine of the first patient (Q), and incubated for 56 days) was filtered on the 18th day after inoculation, and this inoculated into the circulation of rabbit No. 7. This rabbit showed glycosuria in 7 days after inoculation.

Experiment 5. Some of the filtered urine of patient Q, received on January 10, was placed in sterile ampoules, and kept at room temperature. On October 28 rabbits No. 10 and No. 11 were given intravenous injections of 2 cc. of the urine. After 7 days rabbit No. 11 had a trace of sugar in its urine, while rabbit No. 10 first showed a trace of sugar after 11 days. The sugar disappeared from the urine after several days, and reappeared later. So far these rabbits have not shown large amounts of sugar, but in all probability will show greater quantities as the disease progresses.

Experiment 6. Some of the urine of patient Q, received on January 10, was inoculated into serum broth and cultivated for 56 days. A portion of the culture was used for experiment 2, and a portion was reinoculated into serum broth. Some of the subculture was used for experiment 3, and the remainder was reinoculated into serum broth. This subculture was inoculated into fresh serum broth 20 days later, and grown at room temperature under vaseline seal for 6 months, when 0.5 cc. of the culture was injected intravenously into rabbit No. 12. This rabbit had a trace of sugar in its urine 16 days after inoculation.

Experiment 7. Diabetic urine from a second patient (W) was filtered and inoculated into the circulation of 2 normal rabbits in 2 cc. dose. These rabbits (Nos. 3 and 4) showed no glucose in the urine until 28 days after inoculation, and then in only small quantities. Again on the 35th day small amounts of glucose were found. On the 38th day rabbits Nos. 3 and 4 were in a comatose condition and had to be killed. Rabbit No. 4 had a small quantity of urine in the bladder at autopsy, and this showed a slight reaction for glucose with Benedict's solution.

Several of the rabbits, when killed, showed slight congestion of the pancreas, but on sectioning and staining no definite pathologic changes could be demonstrated. There was, apparently, a suggestion of degeneration of the islands of Langerhans, but the alterations were not marked. In rabbits that have been under experiment for longer periods of time microscopic sections of the pancreas and other internal organs may possibly show more convincing evidence of pathologic changes.

The results of these preliminay experiments suggest that *Diabetes mellitus* is caused by a filterable virus; that the disease can be transmitted to rabbits by intravenous administration of a single dose of 2 cc. of the urine of diabetic patients, passed through Berkefeld filters to remove bacteria; that the filtered urine of diabetic patients contains an ultravisible organism which multiplies in serum-broth medium, since cultures 56 days old produced glycosuria in rabbits more promptly and to a more marked degree than freshly filtered urine.

This transmissibility of *Diabetes mellitus* to rabbits, the passage of the disease to other rabbits through the intravenous injection of filtered urine of inoculated rabbits, and the transmission of the disease through the inoculation of serum-broth cultures of the filtered urine of diabetics, indicates the infectious nature of the disease. A number of normal rabbits have been kept under observation, and so far none have been encountered with sugar in their urine. A number of rabbits have been injected with human non-diabetic urine, and, so far, none of these rabbits has developed glycosuria.

The fact that the virus causing *Diabetes mellitus* can be cultivated opens the way for the development of specific prophylactic and therapeutic measures against this disease. Time has not been available to develop this line of investigation, but it is hoped that by bringing the results of this preliminary study to the attention of other investigators work in these several fields will be stimulated.

There is no doubt that systematic study of the blood of normal and inoculated rabbits will aid in the solution of the problem of the etiology of diabetes, and at the same time elucidate the irregularity of the appearance of glucose in the urine of inoculated rabbits.

More detailed study of the pathology of infection in rabbits is also needed to determine whether the changes in the pancreas and other internal organs are similar to those in man. For this study it will be necessary to keep inoculated rabbits under observation for a year or more so as to permit the development of alterations in the pancreas and other internal organs.

The relation of the form of diabetes in children, which is often rapidly fatal, to the slowly progressing disease in adults, may also be elucidated through experiments on animals.

3309

New Colorimetric Method for Determination of Bile Acids in Blood.

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An exhaustive comparative study of the hitherto published methods for the determination of bile acids and salts in the blood demonstrated that they entail a very large loss of these substances by adsorption in the precipitation of the proteins, and in the removal of the bile pigments by charcoal. This fact could have been anticipated, if it is recalled that the bile acids are the most surface-active substances that are present in the blood. We found that by precipitating the proteins with an alcoholic solution of the alkaline $ZnSO_4$-NaOH reagent used in the Hagedorn-Jensen blood sugar method, and by avoiding the use of charcoal, a quantitative recovery (90 to 95 per cent) of bile salts added to blood plasma could be effected. The new color reaction is a modification of the well known Liebermann-Burchard reaction for cholesterol.

The method is as follows: To a mixture consisting of 75 cc. of a freshly prepared 0.45 percent $ZnSO_4$ solution, 15 cc. of 0.1 *N* NaOH solution and 50 cc. of redistilled alcohol, 5 cc. of plasma are added, drop by drop with shaking. The mixture is allowed to stand over night and filtered. The filtrate is evaporated to dryness on the water bath. The protein precipitate is washed on the filter with 50 cc. of boiling water. These hot water washings are used to dissolve out the bile salts from the residue of the alcoholic filtrate, leaving undissolved the bile pigments and some of the lipoids. The filtered aqueous solution is evaporated to dryness, and the residue is washed several times with ether to remove the remaining traces of bile pigments and cholesterol. The remaining dry residue is dissolved in 5 cc. of acetic anhydride, transferred with filtration to a narrow test tube graduated at 5 cc., and 10 drops of the purest concentrated sulfuric acid are added, and the tube shaken. Standards are prepared simultaneously and similarly from known solutions of purified bile salts in acetic anhydride. The clear brown color of the solutions is compared in the usual way in a microcolorimeter after 15 to 20 minutes of standing. By this method from 1 to 10 mg. of added bile salts could be quantitatively recovered from blood, with an error of 5 per cent for the larger quantities, the error increasing to 10 per cent for the smallest quantity of 1 mg. The presence of bile salts could not be demonstrated by this method in normal blood, nor in blood drawn from patients without jaundice or liver disease.

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3310

The Behavior of Electrolytes in Valonia.

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The accumulation[1] of strong electrolytes in living cells presents a problem of peculiar interest. An example of this is afforded by *Valonia macrophysa*. The large multinuclear cell of this marine alga consists of a very thin protoplasmic membrane, inside of which is a large central vacuole filled with sap, and outside of which lies a cell wall imbibed with sea water. It is a very striking fact that the sap inside of this very thin film of protoplasm has a concentration of K about forty times as great as that in the sea water which bathes the outside of the film.[2]

This can be explained if we assume that the protoplasm is impermeable[3] (or nearly so) to K^+ and OH^- but that KOH enters in the form of undissociated molecules. This does not require the assumption that molecules of KOH exist as such in the sea water. We assume rather that K^+ and OH^-, striking the surface of the protoplasm, which is presumably non-aqueous, associate to form KOH which then passes through the non-aqueous layer (for the sake of simplicity we will assume for the moment that the entire protoplasm consists of a single non-aqueous layer[4]) and dissociates in the sap inside. Equilibrium will be reached when as many molecules of KOH enter the external surface in unit time as enter the internal surface.

Since the pH value of the sap is about 5.8, while that of the sea water is about 8.2, it is evident that the concentration of OH^- is about 250 times as great in the sea water as in the sap, and that, in consequence, there must be at equilibrium a much higher concentration of K^+ in the sap than in the sea water, to ensure that as many molecules of KOH enter the internal protoplasmic surface, as enter the external surface. We should therefore expect KOH to enter until the concentration of K^+ in the sap becomes great enough to bring this about; we should then find a much higher concentration of K in the sap than in the sea water, and the accumulation would be explained.[5]

The cell continually produces CO_2, and probably other acids, and if this takes place rapidly as KOH penetrates, the pH of the sap will not rise. We might in that case expect to find $KHCO_3$ and K_2CO_3 accumulating in the sap. Confining the discussion for simplicity to $KHCO_3$, the following suggestion may be made: There may be an exchange of the HCO^-_3 produced inside with Cl^- coming in from the sea water, passing through the protoplasm as ions or as undissociated molecules (CO_2, H_2CO_3, HCl). The distribution of Cl^- might be regulated by the Donnan principle. If K^+ and Na^+ can enter as undissociated molecules which dissociate inside, they are probably to be regarded as diffusible ions in the Donnan sense (since the Donnan principle is not concerned with the manner in which they pass through the membrane, but only with the final result). Even so, if they penetrate very slowly they may be regarded as approximately indiffusible. Since there would appear to be an excess of $K^+ + Na^+ + Ca^{++}$ inside over $K^+ + Na^+ + Mg^{++}$[6]$+ Ca^{++}$ outside, a constraint may be set up across the protoplasm which will cause an accumulation inside of such anions as can enter more rapidly. If we assume that this applies to Cl^- we should expect to find a higher concentration of Cl^- inside than outside and this is actually the case.[7] It is evident that when Cl^- enters, HCO^-_3 must go out in order to preserve the electrical neutrality of the sap.

It is therefore evident that we can picture a mechanism which will account for the penetration of KOH and its accumulation in the sap as KCl. It is possible that KCl[8] penetrates as such, but this would produce no accumulation, since the penetration would cease as soon as the concentration became equal on the two sides. It would seem that the passage of KCl through the protoplasm is very slow, else it would diffuse out more than it appears to do.

It is evident that the production of CO_2 and the accumulation of KCl will raise the osmotic pressure of the sap and cause the cell to grow by absorbing water.[9] Both growth and accumulation of KCl will then depend on the production of CO_2.[10] The concentration of KCl might be expected to remain approximately the same, as long as the cell is growing (since growth and accumulation are both due to CO_2) and this is actually found to be the case.

We should therefore expect that anything which increases the production of CO_2 will increase growth and accumulation, *e. g.*, temperature, light, etc. This might explain the results of Hoagland and his associates[11] who find that light increases the rate of accumulation in *Nitella*. It may be added that this is in harmony with the fact that the most rapidly growing cells are those which produce the most CO_2.

If the cell is much less permeable to undissociated molecules of NaOH than to those of KOH, it is evident that as long as it is growing KCl will accumulate inside rather than NaCl, so that the concentration of NaCl in the sap may be much less than that of KCl (even though the reverse is the case in sea water) and much less inside than outside. This is actually found to be the case in *Valonia macrophysa*.[12] In other organisms where a higher proportion of Na is found we must suppose that the protoplasm is more permeable to it.

If NaCl[8] penetrates as undissociated molecules the concentration inside and outside will tend to become equal, but this penetration may be too slow to make any noticeable change as long as the cell is growing. When growth stops, the penetration of NaCl will tend to equalize its inside and outside concentration more rapidly, and the penetration of NaOH will tend to make Na accumulate inside if the pH value is lower than that of the sea water, but both of these processes may be too slow to be noticeable.

This brief outline is intended only to suggest certain possibilities which are being tested in a variety of ways and which will be discussed in later reports. It may be added that they are in harmony with the results of an extensive series of experiments on *Nitella* and *Valonia*.

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[1] The term accumulation is used to denote a greater concentration inside the cell than outside.

[2] The molecular composition of the sap expressed as per cent of the halide in the sap is Cl+Br 100, K 86.24, Na 15.08, Ca 0.288; that of the sea water expressed as per cent of the halide in sea water is Cl+Br 100, K^{-1} 2.15, Na 85.87, Ca 2.05, Mg 9.74, SO_4 6.26. The sap contains about 1.4 parts per thousand organic matter and the halide content is about 0.6 M as compared with 0.58 M in the sea water.

[3] It is not necessary to assume absolute impermeability but only slow penetration. We know, however, that certain ions, such as Mg^{++} and SO^{-}_{4}, never penetrate into sap from sea water under normal conditions. A considerable variety of evidence indicates that in general undissociated molecules penetrate much more rapidly than ions.

[4] We assume for the sake of simplicity that the protoplasm consists of a single non-aqueous layer but it does not alter the argument if we assume that it consists of an aqueous phase, W, with an outer non-aqueous surface layer, X,

and a similar inner surface layer, Y, since KOH will pass through X and dissociate in W and then associate at the surface of Y, pass through Y, and dissociate in the sap.

[5] The accumulation does not reach equilibrium since as a rule it does not go beyond the point where there is about 40 times as much K in the sap as in the sea water.

[6] This really refers to the Mg in the sea water in excess of SO_4 and takes account of the valence of Mg.

[7] It might be supposed that HCO^-_3 would also be more concentrated inside, but there is a complication due to the fact that the system is not in equilibrium so that the outside is more alkaline and has a higher concentration of HCO^-_3. Osterhout and Dorcas (*J. Gen. Physiol.*, 1925-26, ix, 255) have shown that CO_2 passes rather rapidly through the protoplasm until the concentration of undissociated molecules becomes approximately equal on both sides and that there is more dissociation outside owing to the high pH value of the sea water; hence the higher concentration of HCO^-_3 outside. This complication does not affect HCl which is equally dissociated on both sides. (If the system were in equilibrium with an excess of indiffusible cations inside the pH value inside would be higher than outside and HCO^-_3 would be more concentrated inside, but in both cases at a lower concentration than Cl^-.)

[8] When we speak of the penetration of KCl, NaCl, and NaOH as undissociated molecules we need not assume the existence of such molecules in the sea water, any more than in the case of KOH, since ions striking the surface of the protoplasm may there associate to form molecules and pass as such through the non-aqueous layer.

[9] If the cell wall offers no mechanical resistance we should expect the osmotic pressure inside to be approximately equal to that outside. As a matter of fact there seems to be some mechanical resistance in the case of *Valonia macrophysa* and the osmotic pressure inside is a little higher; the internal osmotic pressure is due almost entirely to KCl and NaCl and the concentration of halide is a little higher inside (see footnote 2).

[10] In case the increase in osmotic pressure and consequent growth is due to such a substance as sugar this will not apply.

[11] Hoagland, D. R., and Davis, A. R., *J. Gen. Physiol.*, 1923-24, vi, 47. Hoagland, D. R., Hibbard, P. L., and Davis, A. R., *J. Gen. Physiol.*, 1926-27, x, 121. We should assume that light acts indirectly in this case by providing for the production of CO_2.

[12] Experiments during the last five years on the penetration of ammonia into *Nitella* and *Valonia* indicate that it penetrates as NH_3 or as undissociated NH_4OH and accumulates as NH_4Cl, as described above for KOH and NaOH, and that it penetrates much more rapidly than KOH and NaOH.

3311

Changes in the Respiratory Quotient Produced by Subcutaneous Injection of Dioxyacetone and Glucose.

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Evidence on the availability of dioxyacetone and glucose by the animal organism has led to opposing conclusions. Rabinowitch[1] and Mason[2] have found that dioxyacetone is more readily oxidized than glucose. Ringer and Frankel[3] have shown that in diabetic dogs dioxyacetone is synthesized to glucose. Campbell, Fletcher, Hepburn and Markowitz,[4] obtaining similar results, concluded that dioxyacetone presents no advantage to the organism, since it must be converted to glucose, prior to oxidation. In the present experiments the availability of these two substances has been studied after subcutaneous injection.

Fifteen experiments were performed on a trained dog, in which 10 grams of the substance studied, dissolved in 10 cc. of warm water, were injected subcutaneously. Seven experiments were performed using dioxyacetone, seven using glucose, and one employing sucrose. The dog had been trained to lie quietly with a leak-proof mask on its head, the expired air being collected through Sadd valves into a spirometer. Air was collected before injection, a second sample of air was taken in the first 3 or 5 minutes after injection, and the subsequent collections of air were taken in 5 to 10-minute periods thereafter. An interval of 3 minutes between collections was required for the removal of samples of the collected air from the spirometer. These air samples were kept in glass sampling tubes over mercury and analyzed for CO_2 and O_2 by means of the Haldane-Henderson apparatus.

In the first 5 minutes after injection of dioxyacetone, 5 experiments, starting with an average respiratory quotient of .80, yielded a respiratory quotient of .92, while in 15 minutes the respiratory quotient rose to 1.03. The remaining 2 dioxyacetone experiments, starting with a higher respiratory quotient, due to the feeding of sucrose on the night prior to the experiment, showed an average pre-injection value of 1.14. Five minutes after injection the respiratory quotient was 1.15. After 15 minutes it was 1.17, and at the 1/2 hour was 1.23. Fifty-five minutes after injection the respiratory quotient was found to be 1.31.

Five of the 7 glucose experiments, starting with a basal respiratory quotient of .73, rose in 15 minutes to .76, in 27 minutes to .77, and in 50 minutes to .80. Two glucose experiments, starting with an average respiratory quotient of .87 rose to 1.00 in 27 minutes. The 40th minute respiratory quotient was .98, and in the 55th minute was .97.

Following the same technique, a control experiment performed with sucrose yielded no change in the respiratory quotient over the period of 1 hour.

CONCLUSIONS.

Dioxyacetone, in contradistinction to glucose, caused an immediate and considerable rise in the respiratory quotient, the average increase being .19 for the first 15 minutes. Glucose caused a rise of only .04 in 15 minutes and .07 in one hour. These results indicate that dioxyacetone is more readily available than glucose when administered subcutaneously.

In experiments now in progress we are studying the rates of absorption of glucose and dioxyacetone after subcutaneous injection. If these experiments do not indicate a slower absorption of glucose, as compared with that of dioxyacetone, it might be concluded that dioxyacetone is more readily oxidized in the normal animal. On the other hand, if the data show that dioxyacetone is absorbed more rapidly than glucose it would manifestly be impossible to ascribe a more rapid rise in respiratory quotient in the case of dioxyacetone to its greater susceptibility to oxidation, unless quantitative evidence on the rates of absorption, and the rates of oxidation, were at hand.

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[1] Rabinowitch, I. M., *J. Biol. Chem.*, 1925, lxv, 55.

[2] Mason, E. H., *J. Clin. Inv.*, 1926, ii, 521, 533.

[3] Ringer, A. I., and Frankel, E. M., *J. Biol. Chem.*, 1914, xviii, 233.

[4] Campbell, W. R., Fletcher, A. A., Hepburn, J., and Markowitz, J., *J. Pharmacol. and Exp. Ther.*, 1926, xxvii, 258.

3312

Studies upon Calcification in Vitro: I. Effect of Reaction on Calcification.

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Shipley, Kramer and Howland[1] have shown that when a section of a rachitic bone is immersed in an inorganic solution containing calcium and phosphorus, calcification occurs in the provisional zone of cartilage. This calcification resembles that occurring *in vivo* following the administration of an antirachitic agent to a rachitic animal. These authors have also demonstrated that calcification occurs only when the product of the concentration of the calcium and inorganic phosphorus exceeds 35. The significance of the Ca × P product in the serum of patients with infantile rickets, and animals suffering from experimental rickets, has been emphasized by Howland and Kramer[2] and confirmed by others.

The present investigation is an extension of the study of calcification *in vitro,* and deals with the effect of reaction (pH) on this process. The procedures employed were essentially the same as described by Shipley, Kramer and Howland, with the exception of a few technical variations. Whereas they used the McCollum No. 3143 diet in producing a rachitic condition in rats, we have employed the Steenbock diet No. 2965. The latter diet produces a very severe type of rickets within 17 days, and the sections obtained from these animals are very well adapted to an investigation of this kind, since the metaphysis is very wide and the cartilaginous zone very pronounced.

In order to study the effect of reaction, the calcium-phosphorus concentration was kept constant, and the pH varied from the acid to the alkaline sides of the physiological range. The results may be summarized briefly as follows:

1. The optimum pH for *in vitro* calcification, when the product of Ca × P is 50, was found to be 7.25 to 7.30.

2. Under these conditions calcification may appear after two hours' incubation.

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3. Calcification occurs only in places in the zone of provisional cartilage at a pH of 7.1 to 7.2.

4. Slight calcification may occur at a pH of 7.0 to 7.1.

5. No calcification occurs in solutions more acid than pH 7.0, and the sections appear swollen and tenacious.

6. In solutions more alkaline than pH 7.35, calcification occurs, but the initial pH usually changes to the optimum pH of 7.25 or 7.3 over night.

7. Sections left in acid solutions for from 2 to 6 hours and later transferred into solutions in which the concentrations of calcium, inorganic phosphorus and hydrogen ions are optimal, fail to calcify; whereas sections transferred from solutions of low Ca × P product into solutions of a higher product may calcify if the pH is optimal.

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[1] Shipley, P. G., Kramer, B., and Howland, J., *Biochem. J.*, 1926, xx, No. 2, 379.

[2] Howland, J., and Kramer, B., *Monatsschr. f. Kinderheilk*, 1923, xxv, 279.

3313

The Gasometric Determination of Potassium.

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We have used the constant volume apparatus of Van Slyke and Neill in the estimation of potassium in solutions whose inorganic composition approximates that of serum. The potassium is precipitated as the potassium-sodium cobalti-nitrite compound, essentially as in the original method of Kramer and Tisdall.[1] The precipitate is decomposed with sodium hydroxide as suggested by Clausen,[2]* and the nitrogen of the sodium and potassium nitrite is liberated by adding a solution of urea and sulphuric acid.[3]† The amount of nitrogen is then determined in the constant volume apparatus. It is possible to determine from 0.07 to 0.45 mg. potassium, with an error not exceeding 0.01 mg.

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* $K_2NaCo(NO_2)_6 + 3\ NaOH = 2\,KNO_2 + 4\,NaNO_2 + Co(OH)_3$.

† $CO(NH_2)_2 + 2\,HNO_2 = 2\,N_2 + CO_2 + 3\,H_2O$

One cc. of the potassium solution is measured into a 15 cc. graduated centrifuge tube, containing 1 cc. of water. One cc. of the sodium-cobalti-nitrite reagent is added slowly, drop by drop, and the solution thoroughly mixed after each drop. At the end of 45 minutes, the volume is made up to 5 cc. with water and the contents mixed again and centrifuged for one-half hour at a speed of about 1800 revolutions per minute.

All but 0.3 cc. of the supernatant fluid is pipetted off so as not to disturb the precipitate. Five cc. of water are allowed to run in slowly. The stream is controlled in such a way that the added water is thoroughly mixed with the residual reagent and the precipitate is not disturbed. The tube is centrifuged for 5 minutes. The procedure is repeated twice, making three washings in all. In the third washing, the water is added in such a way as to gently agitate the upper portion of the precipitate. It is emphasized that the supernatant fluid from the last washing must be colorless. After this has been pipetted off, 0.7 cc. of approximately normal sodium hydroxide is added and the precipitate is thoroughly mixed with a fine glass rod. The tube is heated in a boiling water bath for about 5 minutes, after which a brownish flocculent precipitate of cobalt hydroxide settles on the bottom of the tube. The solution is cooled and transferred to the cup of the Van Slyke gas apparatus. The remainder of the precipitate is washed into the cup, with 2 cc. of 10 per cent urea solution. One cc. of approximately 8 N sulphuric acid is led in, after which a vigorous evolution of gas takes place. The mercury is lowered almost to the bottom of the 50 cc. bulb, the apparatus is sealed, and the level of the mercury then lowered to the 50 cc. mark. After shaking for about 5 minutes, the mercury is raised again so that a space of several cc. is left between the water meniscus and the 2 cc. mark. Three cc. of approximately 10 N sodium hydroxide is permitted to flow gradually into the chamber. The apparatus is sealed again and the carbon dioxide is absorbed. The gas volume is reduced to the 2 cc. mark and the pressure and temperature are read. To obtain the pressure which is exerted by the nitrogen liberated, subtract a blank pressure which is obtained on 1 cc. of approximately normal NaOH treated in the same manner as the unknown.

The pressure is converted into millimols of nitrogen by using the last column of Van Slyke's Table III.[1] Since 1 cc. of solution was taken for analysis, and the factors are for 2 cc. samples, the resulting value is multiplied by 2 to obtain the millimols per liter of solution. Since the quantity of nitrogen evolved in the reaction is twice the amount present in the nitrite, this value is divided by 2. The number of milligrams of nitrogen so calculated is multiplied by 0.9286 (the ratio of potassium to nitrogen in the $K_2NaCo(NO_2)_6$ precipitate) in order to obtain the value for potassium in milligrams per 100 cc.

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[1] Kramer, B., and Tisdall, F. T., *J. Biol. Chem.*, 1921, xlvi, No. 2, 339.

[2] Clausen, S. W., *J. Biol. Chem.*, 1918, xxxvi, 479.

[3] Frankland, P., *J. Chem. Soc.*, 1888, liii, 364.

3314

The Metabolism and the Respiratory Quotient of Excised Renal Tissue.

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The respiratory quotient of the excised renal tissue of the rat was determined by the Warburg-Barcroft manometers. The accuracy of the method was judged by duplicate quotients, performed with the same tissue, at the same time. The results are recorded in Table I, columns 1 and 2. The average of each pair is given in the third column, and the numerical difference in the fourth. The series was consecutive, and only those observations discarded in which technical error could be demonstrated. Observation 7 is excluded from the averages; a technical error was probable, but not proved. With this exception, the mean difference was 0.027, and the error ±0.014.

The data include observations on well-fed, as well as fasting rats, with or without glucose in the fluid. Variations in the results would therefore be expected. The lowest observation was 0.705, which happens to be the theoretical quotient for fat. The highest was 0.949, which does not exceed the level of the exclusive oxidation of carbohydrate. In 19 out of 25 observations the quotients were between 0.750 and 0.899. Thus all the experiments fall within the limits which we should expect to find in the intact organism, even when there is no reason to suspect the formation of fat from carbohydrate, or the reverse. The results imply that excised tissue,

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TABLE I.

Duplicate One-hour Quotients; Glucose in Medium.

(1)	(2)	Average (3)	Difference (4)
Fed.			
0.820	0.813	0.817	0.007
0.847	0.823	0.835	0.024
0.760	0.788	0.774	0.028
0.688	0.721	0.705	0.033
0.822	0.802	0.812	0.020
0.845	0.872	0.858	0.027
0.907	0.774		
0.894	0.836	0.865	0.058
0.869	0.819	0.844	0.050
0.796	0.768	0.782	0.028
0.792	0.800	0.796	0.008
Fasted.			
0.861	0.876	0.868	0.015
0.949	0.938	0.944	0.011
0.887	0.843	0.865	0.044
Average			0.027

though deprived of nerve connections and circulation, utilizes the same proportion of foodstuffs as the intact organism. Evidence of the conversion of one food-material into another was lacking.

Our earlier experiments raised the question, whether or not excised renal tissue could make use of the glucose offered it in Ringer solution. To answer this query, simultaneous observations were made, with and without glucose in the medium. The results are recorded in Table II. With glucose, the quotients were invariably higher, and the difference far exceeded the experimental error. The

TABLE II.

Simultaneous Respiratory Quotients, with and without Glucose in Medium. Two-hour observations.

Without Glucose	With Glucose	Difference
Fed.		
0.830	0.900	+0.070
0.824	0.872	+0.048
0.816	0.867	+0.051
0.820	0.884	+0.064
Av. 0.823	0.881	+0.058
Fasted.		
0.811	0.898	+0.087
0.805	0.878	+0.073
Av. 0.808	0.888	+0.080

average increase was 0.058. In two experiments the same procedure was carried out after 3 days of fasting. The results did not differ from those obtained after food. In either case the presence of glucose caused an increase in the quotient, which may be attributed to the oxidation of sugar.

CONCLUSIONS.

1. It is shown by complete double respiratory quotients that in the case of renal tissue, the error of the Warburg-Barcroft manometers is, on the average, ±0.014.
2. The quotients were all between 0.7 and 1.0, and are fully accounted for by the oxidation of foodstuffs, without postulating the transformation of one into another.
3. As evidenced by the rise in the respiratory quotient, isolated tissue can oxidize glucose offered it in solution.

3315

Removal of Inhibiting Effects on Nitella of Certain Buffer Mixtures and Acids.

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From the Laboratories of The Rockefeller Institute for Medical Research.

Living cells of *Nitella* (collected in New York in autumn) were exposed[1] for 10 minutes to (1) M/150 phosphate buffer solution at pH 5.5, (2) 5×10^{-5} M phosphoric acid[2] (at about pH 4.3), and (3) 5×10^{-5} M hydrochloric acid (at about pH 4.2), and then placed in a solution of brilliant cresyl blue.[3] The rate of penetration of dye into the vacuole, as compared to that of the control (cells transferred from tap water into the dye solution), was found to decrease about 50 per cent in (1), 37 per cent in[4] (2), and 25 per cent[5] in (3).

This inhibiting effect was not removed when cells, previously exposed to the solutions above mentioned, were washed for ½ minute either in M/150 phosphate buffer solution at pH 7.7, or in M/150 borate buffer solution at pH 7.7 containing 0.02 M sodium chloride. In the latter case there was a greater inhibiting effect. The inhibiting effect of hydrochloric acid and phosphoric acid was very slightly reduced while that of the phosphate buffer mixture at pH 5.4 was not changed when cells previously exposed to these solutions were washed in M/150 borate buffer solution at pH 7.7 for ½ minute.

The inhibiting effect, however, was completely removed when cells previously exposed to the solutions mentioned were treated in any of the following ways:

(a) By placing the cells in the dye solution made up with M/150 phosphate buffer mixture. The same result was obtained whether the dye solution was stirred or not.

(b) By placing the cells in the dye solution made up with M/150 borate buffer mixture, containing either 0.02 M sodium chloride or 0.01 M magnesium chloride.

(c) By washing the cells for ½ minute in 0.01 M magnesium chloride dissolved in M/150 borate buffer mixture at pH 7.7 and then placing them in the dye solution made up with borate buffer mixture.

Regarding the above experiments the following suggestions may be made:

(1) Magnesium chloride either displaces from the protoplasm the substances causing an inhibiting effect, or it acts on the protoplasm in such a way that it nullifies their effect (it may be that it decreases the viscosity of the protoplasm).

(2) The dye is affected by NaCl, and by phosphate buffer mixture, (in all probability chiefly by the base cations) in such a way that it is able to mask the effect of sodium chloride on the protoplasm.

(3) It is uncertain whether the dye is affected by magnesium chloride in the same manner as by sodium chloride as described in (2).

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[1] In all the experiments the solutions to which the cells were exposed before placing in the dye were not stirred.

[2] The pH value of phosphoric acid seemed a little higher than that of hydrochloric acid, but this difference is not sufficient to account for the difference in the behavior of the two acids toward protoplasm.

[3] The following holds for every experiment unless otherwise stated: The dye solution was 0.00014 M, made up with M/150 borate buffer mixture at pH 7.7. The dye solution was stirred at the rate of one revolution per second. The determination of the dye in the vacuole was made colorimetrically after the cells had been placed in the dye solution for ½ minute.

[4] The experiments with the same lot of *Nitella* showed that at pH 4.8 there is still 20 per cent decrease with phosphoric acid, while there is none with hydrochloric acid. (Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 271.)

[5] This result is different from that in the writer's paper (PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 54.), where at pH 4 there was no inhibiting effect with hydrochloric acid while there was about 20 per cent decrease with phosphoric

acid. This difference is due to the fact that cells were collected in two different localities. In the former case the cells, which were collected in New York, were more sensitive than in the latter (cells collected in Cambridge). This is shown by the fact that when sufficiently low concentrations of these acids were used (0.000025 M at pH 4.8) with New York *Nitella* (see footnote 4) the same result was obtained as with Cambridge *Nitella* (using acids at pH 4.)

3316

Exit of Dye from Nitella with Different Initial Concentrations of Dye in the Vacuole.

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Living cells of *Nitella* were placed in brilliant cresyl blue solution for different lengths of time, so that the concentration of dye[1] (DB plus DS) in the vacuole varied from 0.000035 M to 0.0028 M (cell wall only very slightly stained). When such cells were placed in the M/150 phosphate buffer solution at pH 5.4 (solution changed and stirred), the rate of exit of dye from the vacuole was found to be about the same between 0.000035 M and 0.00014 M. Above the latter concentration it decreased as the concentration increased. There is also a decrease in the velocity constant, k, calculated from the equation $dx/dt = k(a-x)$, where a equals the initial concentration of dye in the vacuole, and x equals the amount of dye that has disappeared from the vacuole at time t. The pH value of the sap was about the same as that of the normal (determined after the dye has come out of the vacuole).

It may be assumed that the conditions, either in the vacuole or in the protoplasm or both, change as the concentration of dye increases. This change may be due to the production of a substance which (1) combines with part of DB to form a non-penetrating compound (other than DS), of the same color as DB and DS, which breaks down slowly to form DB during the exit of the dye, or (2) otherwise changes the concentration of DB.

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[1] It is assumed that the dye exists in two forms, free base (for convenience called DB) and salt (for convenience called DS). DB penetrates the living cell very rapidly while DS penetrates so slowly that its penetration in this case is negligible. As DB enters the vacuole a certain proportion of it changes to DS. Though we measure the amount of DB plus DS in the vacuole, this method is justified because there is always a definite ratio of DB to DS, and because both DB and DS are of the same color. (*Cf.* Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 75.)

3317

On a Specific Substance of the Cholera Vibrio.

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Differences in opinion (Levaditi,[1] Prausnitz[2]) as to the alcohol solubility of the specific substances of the cholera *Vibrio* called for an experimental investigation of the subject.

While we were unable to detect such substances in extracts made with ether or hot absolute alcohol, they were present in 75 per cent alcoholic extracts of the bacilli, previously washed with saline, and extracted with ether and hot absolute alcohol. On cooling, a sediment separated from the solution which reacted with anti-cholera immune serum, up to dilutions of 1-500,000 of the antigen. This product in a 1 per cent solution gave distinct protein reactions. On hydrolysis with dilute hydrochloric acid, the solution reduced Fehling's reagent and gave a crystalline osazone. During the hydrolysis the fluid became turbid, and an insoluble product settled. The hot 75 per cent alcoholic extract was put through a Berkefeld filter, and the solution of the sediment, which appeared on cooling, was injected into rabbits. After 3 injections of 2 mg. each of the substance, precipitating and agglutinating antisera were obtained.

For further separation of the specifically reacting substance, the extraction was made first with a smaller, and then a larger volume of hot 75 per cent alcohol, and the sediment from the second extract only was used. To a 10 per cent solution, four volumes of N/10 NaOH were added, and after centrifuging, the solution was precipitated with alcohol. The precipitate was then redissolved in water, neutralized, some Na_2CO_3 added, centrifuged, and the solution precipitated with alcohol, after acidifying with acetic acid.

The precipitate obtained is a white powder, soluble in water. That the specific substance contains carbohydrates is indicated by the formation of reducing sugar upon hydrolysis. Also the insoluble substance mentioned above separated, after heating with dilute acid. A 1 per cent solution gave no biuret reaction.

The purified product had very slight antigenic activity, if any. With immune sera it reacted in a dilution of 1-500,000. In higher concentrations precipitation appears immediately, leading to the formation of heavy flakes. An analysis of this product gave the following values, calculated for ash free substance, C. 49.05; H. 7.17; N, 4.34; P, 1.67; no S. Other preparations gave similar figures.

An explanation of the results of the immunizations could be given, if there exists in the dilute alcohol extract a complex antigen containing protein, and the specifically reacting non-immunizing substance.

Substances apparently similar to that described were found in another *Vibrio,* and in bacilli of the typhoid group (in collaboration with Dr. J. Furth).

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[1] Levaditi, C., and Muttermilch, S., *Compt. rend. Soc. Biol.,* 1908, lxiv, 406, 844, 1151; *ibid.,* xlv, 26.

[2] Prausnitz, C., *Centr. Bakt.,* 1911, lix, 434.

3318

On the Alcohol Soluble Specific Substance of B. Smegmae.

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It is of interest to find out if the close serological relation between the alcohol soluble substances of tubercle bacillus and that of *B. smegmae* is connected with a similarity as to their chemical characteristics, as far as they can be established.

The smegma strain used was the same as employed in previous work in this laboratory. It is not impossible that between the different strains of smegma bacillus there is a variation as to their serological properties. The methylalcohol extract was obtained after previous extraction of the dried bacilli with ether (twice) and ethylalcohol (twice). The immunserum was obtained from rabbits injected with suspension of the bacilli. The titer of the serum in the complement fixation was 1:300 with the bacillary suspension and also with alcohol extract.

In the Castellani experiment made with tuberculosis and smegma immunsera, no difference was found between the corresponding extracts, as they absorbed the antibodies for each other from both of the sera.

The specific substance is soluble in ethylalcohol and in ether, and insoluble in acetone, like the specific substance of alcoholic extract of tubercle bacillus. The crude methylalcohol extract was purified with acetone. The extraction of the evaporated extract, with acetone, seemed to enhance the potency of the preparation; the antigen unit of the crude extract 0.00015 mgm. after extraction with acetone, and, solution in ether was 0.00005 mgm. Preparations obtained after two acetone precipitations of the ether solution of the substance lost something of their potency. They were markedly more potent than the original crude methylalcoholic extract (antigen unit 0.00015 mgm.). None of the acetone solutions has shown specific effect. In most of the preparations the antigen unit is somewhat smaller than the antigen unit of the corresponding preparation made from tubercle bacillus. The difference might be caused by the difference in sera used. One preparation, the ether solution, after the first acetone treatment, is markedly more potent than the others. It is not known to what factor is due the loss of potency of the preparations in the course of repeated precipitation by acetone.

The smallest amount of the crude methylalcoholic extract giving complement fixation is nearly the same, using Serum 597 II, obtained with a fast-growing avirulent strain of tubercle bacillus. It is eight times higher with Serum R obtained with a virluent strain of *B. tuberculosis.* As to the significance of this difference we refer to another publication in this PROCEEDINGS.[1] The serum obtained with the smegma bacillus, with the antibodies corresponding to the alcohol soluble antigen, is nearer the Serum 597 II, than to the Serum R obtained with a virulent strain.

The P. content of various purified preparations varies between 1.3 to 1.8 per cent, and the N content of a preparation purified with acetone but not washed thoroughly with water, was 1.4 per cent. The content of fatty acids (ether soluble substances after hydrolysis) of a preparation purified with acetone was 40 per cent.

The chemical characteristics of the extracts of smegma bacillus reported show close resemblance to the extracts of *B. tuberculosis.* The chemical study of the extracts of smegma bacillus is not sufficiently advanced to enable us to estimate the significance of the differences between the two extracts.

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[1] Dienes, L., and Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 27.

3319

Hypoglycemia Induced by Sodium Selenite.

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Since the discovery of insulin (F. G. Banting and C. H. Best[1]), a number of organic compounds, such as guanidine (Collip,[2] Dubin and Corbitt[3]), adenine sulphate and xanthine (Dubin and Corbitt[3]), ergotamine (Lesser and Zipf[4]), 2 thio, 4 or 5 amino-ethylglyoxaline, a sulphur derivative of histidine (MacDonagh[5]), choline (Junkersdorf and Kohl[6]), and dihydroxyacetone (Rabinowitch,[7] Mason[8]), have been found to possess the property of lowering blood sugar. As for inorganic compounds, potassium chloride, according to Kyelin and Engel,[9] induces a slight fall in blood sugar, while calcium chloride, according to Barath,[10] causes a slight rise. Elias, Popescu-Inotesti and Radoslav[11] report a fall in blood sugar in rabbits and dogs induced by both mono and disodium phosphate. Brugsch and Horsters[12] observe that sodium phosphite also diminishes blood sugar.

We wish to report another inorganic compound, sodium selenite, Na_2SeO_3, that induces hypoglycemia. Although selenium is believed to behave pharmacologically like arsenic, it has been shown by Van Dyke[13] that sodium arsenite produces hyperglycemia.

The extent of the hypoglycemia induced by sodium selenite was determined in rabbits that received no food except water for a period of 18 to 24 hours prior to injection of the selenium compound. Blood sugar was determined by the method of Shaffer and Hartmann.[14] The dose administered subcutaneously was 2.5 to 7 mg. per kilo. The minimal lethal dose proved to be 4 mg. per kilo on a diet low in carbohydrate.

The degree of hypoglycemia was in direct proportion to the quantity of the selenium salt injected. Thus, with a small dose, 2.5 mg. per kilo, the normal blood sugar of the animal remained unchanged for 14 hours, when it dropped from 0.118 per cent to 0.085 per cent. With a larger dose, 6 mg. per kilo, the blood sugar dropped from 0.130 per cent before injection to 0.072 per cent the first hour, and to 0.045 per cent the third hour after injection.

The effect of sodium selenite is shown in one of the experiments cited below:

Weight of rabbit, 2,145 grams. Dose: 6 mg. of sodium selenite per kilo, injected subcutaneously at 2:30 p. m. Animal starved 18 hours prior to injection.

Blood Sugar.

Before injection,	1.050 per cent.
1 hour after injection,	0.087 per cent.
2 hours after injection,	0.048 per cent.

The rabbit went into convulsions at 4:45 p. m. The convulsive movements were somewhat similar to, but not as violent as those observed in strychnine poisoning, but different from those seen in insulin toxemia. The animal died at 6:30 p. m.

This hypoglycemia reflects the efforts of the organism in the direction of detoxication. The organism reduces soluble selenite to free selenium, which in turn is non-toxic. Levine [15] has shown that the reduction of sodium selenite to the free element takes place *in vitro* at 37° C., in the presence of a slightly alkaline solution containing glucose, fructose or galactose, or in a solution slightly acidified with lactic acid. He has further shown that the reduction *in vivo* is partly non-enzymatic and partly the result of a reducing enzyme, the action of which is accelerated by glucose. After histological examination of the tissues stained with eosin and hematoxylin, some organs, especially the liver, show profuse deposits of chocolate colored granules of selenium.

Glycogen in the liver suffers a decrease as a result of the injection of sodium selenite. We have found that the administration of glucose protects the liver cells from degeneration, and prolongs the life of the animal, if selenite has been given in very large doses, and may save it even from death, when the selenite is given in minimal lethal doses. Animals on a diet high in carbohydrate withstand the toxic effects of the selenium salt better than those on a low carbohydrate diet. The experiments of Cathcart and Orr[16] also lead to the same conclusion.

Besides the reduction of selenite to free selenium, another process of detoxication goes on simultaneously, namely, alkylation of selenium, and subsequent expulsion through the respiratory tract of the volatile methyl selenide formed.[17] Rabbits or dogs receiving sodium selenite subcutaneously expire methyl selenide, which has a characteristic and unmistakable odor, detectable in the breath two to three minutes after injection. The odor continues to be present in the expired air for many hours, if the animal survives the dose.

Subcutaneous injection of sodium selenite into the rabbit or dog causes complete withdrawal of free hydrochloric acid from the gastric contents. The probable purpose of this withdrawal of acid is to increase the hydrogen ion concentration of the blood in order to aid the process of glycogenolysis. That the alimentary tract does influence acid-base balance in the blood is shown by the experiments of Tingl,[18] who demonstrated an increase in blood alkali in the course of digestion.

In the light of our experimental findings, we advocate administration of glucose and the use of diets high in carbohydrate in cases of poisoning with selenite. Tellurium compounds behave in the body similarly to selenium. Since it has been shown that glucose detoxicates sodium tellurite by reduction to free tellurium,[19] we also recommend like treatment in poisoning with tellurites.

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3320

On Specificity of Opalina in the Frog.

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From the Zoological Laboratory of Rutgers University, New Brunswick, N. J.

It is well known that the ciliate, *Opalina,* parasitic in the rectum of the leopard frog, *Rana pipiens,* and in the pickerel frog, *R. palustris,* occurs but rarely or not at all in the closely allied adult green frog, *R. clamitans,* and bull frog, *R. catesbiana.* Tadpoles of *R. clamitans* have been found by us to be almost universally parasitized.[1] Experiments by the first author were conducted to determine if *Opalina* would live and reproduce when transferred directly from the rectum of *R. pipiens* to that of the two genera of green frogs.

The frogs used in these experiments included *R. pipiens, R. clamitans,* and *R. palustris,* collected within 70 miles of New Brunswick, N. J., and *R. catesbiana* from New Orleans, La. The parasite employed in these transfers was *O. obtrigonoidea.* Removal of the rectal contents was accomplished with the aid of a fine glass pipette introduced through the cloaca of the host after previously relaxing the surrounding muscles by the action of M/8 magnesium chloride for 20 minutes. A pipette full of Ringers solution was first forced into the rectum before withdrawing the contents for examination or for introduction into a second host. The experiments were carried on from October to late May of the following year. The green frogs and bull frogs used in these experiments were examined in each instance prior to inoculation. One specimen of *clamitans* was found to harbor a heavy infection of *Opalina,* due, possibly, to contamination from close proximity to *pipiens* and their faeces, since the former frog does not normally harbor these parasites.

There follows the inoculations made together with the source of parasites used: *R. clamitans;* from *pipiens* 5, from *palustris* 2, and secondary transfers from artificially infected *clamitans* and *catesbiana,* 1 each. *R. catesbiana;* from *pipiens* 3, from *palustris* 3, and from artificially inoculated *clamitans* 1.

Reëxaminations were made of these frogs at intervals of approximately one month with the following results:

R. clamitans: Five showed no infection after 1 month. Good cultures of *Opalina* were obtained from 2 frogs 162 and 174 days respectively after inoculation from *pipiens.* One gave a good culture 16 days after inoculation from *palustris,* and one showed a fair culture 82 days after secondary inoculation from an artificially infected *catesbiana.*

R. catesbiana: Four showed no infection after 1 month. One gave a good culture 35 days after inoculation from *pipiens,* but none at 71 days. One showed a fair culture of *Opalina* 105 days after inoculation from *pipiens.* In 3 frogs inoculated from *palustris,* and one secondary inoculation, from an artificially infected *clamitans,* the parasites failed to become established.

CONCLUSIONS.

Metcalf[1] has suggested that since *Rana clamitans* and *R. catesbiana* remain for more than one year in the larval condition, adjustment by *Opalina* to the adult stage of the host has not been necessary, infection taking place directly from each generation of tadpoles to the succeeding one. The presence of *Opalina*, however, in toads and in other adult *Anura* which metamorphose in less than one season throws doubt upon this explanation. The occurrence of *Opalina* in an occasional specimen of *R. clamitans*, and the successful propagation of this parasite for 174 days in *R. clamitans* and for 105 days in *R. catesbiana* indicate that *Opalina* may be in the process of adjustment to these two adult hosts. Additional support of this hypothesis is the fact noted by Metcalf that *R. catesbiana* introduced into Hawaii, and from there imported into California, harbored the parasite.

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3321

Some Effects of Alveolar Carbon Dioxide Tension on the Carotid and Femoral Flow of Blood.

ROBERT GESELL AND DETLEV W. BRONK.

From the Department of Physiology, University of Michigan, Ann Arbor, Mich.

The importance of volume-flow, as well as composition of the blood, to the transport of oxygen and carbon dioxide and to respiratory control, led to the development of the electrometric method of recording volume-flow of blood, and its use in the study of disturbances in respiratory equilibrium. This report deals briefly with the effects of artificially produced changes in the alveolar concentration of carbon dioxide.

The volume-flow in the carotid and femoral arteries was continuously recorded, along with mean blood pressure and pulmonary ventilation. Room air and carbon dioxide (4 to 12 per cent) in room air were administered with re-breathing tanks. In some experiments the gases were administered by natural respiration, and in others pneumothorax was established, and the gases administered by closed circuit artificial ventilation.

The administration of carbon dioxide by either method elicited, in general, an increased flow of blood through the carotid artery, followed by a decreased flow on re-administration of room air. The same administration elicited a decreased femoral flow of blood, which increased on re-administration of room air.

Changes in artificial pulmonary ventilation with room air were accompanied by corresponding changes in volume-flow of blood. Increasing pulmonary ventilation above normal resulted in decreased carotid flow, and increased femoral flow. Decreasing ventilation produced reverse effects.

Artificial control of blood pressure showed the same directional changes in volume-flow of blood, in the absence of changes in the driving head of pressure.

The results suggest that the circulatory adjustments occurred in favor of the brain. They illustrate the importance of the factor of acidity in the control of circulation, as well as respiration. The fact that the respiratory and circulatory centers are both highly sensitive to changes in acidity agrees with the common and coördinated function of both.

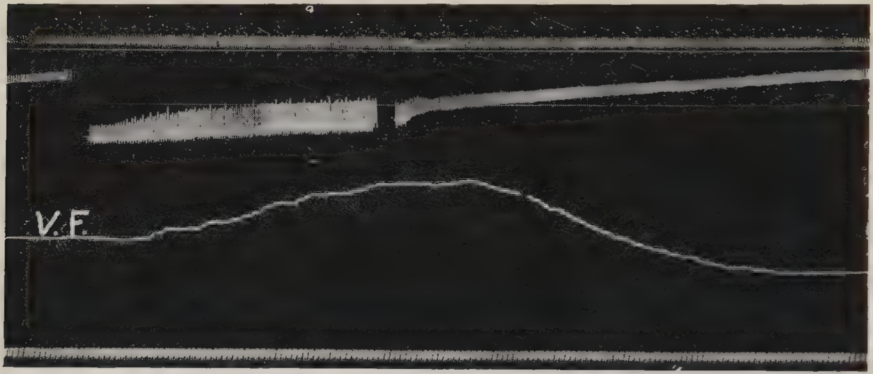

FIG. 1.

Record showing the effects of administration of 12 per cent carbon dioxide on pulmonary ventilation and carotid flow of blood. The flow increased from 47 cc. per minute to 62 and decreased to 41. The mean blood pressure was maintained constant by artificial means.

3322

Low Alveolar Oxygen Pressure, Sodium Cyanide and the Carotid and Femoral Flow of Blood.

DETLEV W. BRONK AND ROBERT GESELL.

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Methods of study similar to those described in the preceding paper on the effects of variations in alveolar carbon dioxide were used.

The administration of low oxygen by either natural or artificial ventilation elicited an increased carotid flow of blood, followed by a decrease on re-administration of room air, and a decreased femoral flow, which increased on subsequent administration of room air. Variations from this response were slightly more common than variations with the administration of carbon dioxide. An initial decrease in carotid flow, preceding the increase, and a subsequent decrease in flow below normal, on re-administration of room air, were not infrequent. Changes in flow were more abrupt, and more fluctuating, than those produced by carbon dioxide. This peculiarity, however, was frequently missing. A response to low oxygen may be identical to a response to high carbon dioxide.

Intravenous injection of sodium cyanide produced a general increase in carotid flow of blood, and a decrease in femoral flow. An initial decrease in carotid flow preceded the increase.

When the mean blood pressure was maintained approximately constant by artificial regulation, the same directional changes in flow obtained.

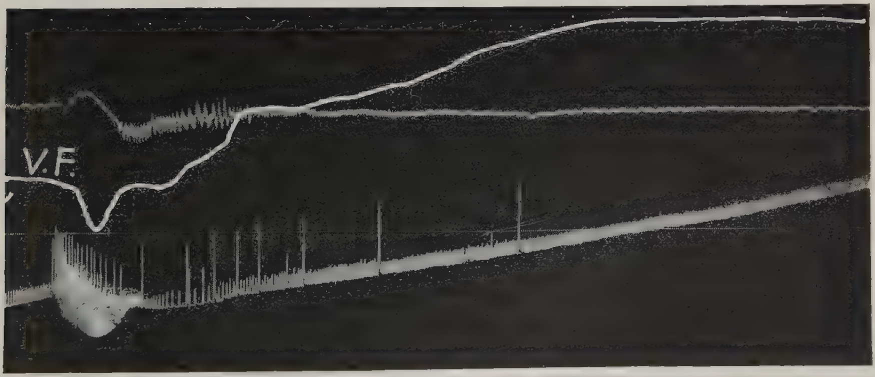

FIG. 1.

Record showing the effects of intravenous injection of sodium cyanide on mean blood pressure (above), carotid flow of blood and pulmonary ventilation (below).

The similarity of circulatory response to low oxygen and sodium cyanide suggests like chemical disturbances in and on the opposite sides of the neurone membranes of the circulatory centers involved in the regulation of circulation.

The inverse relation of carotid and femoral flows suggest again that circulatory adjustments occurred in favor of the brain.

Likewise, the similarity of the circulatory response to the administration of carbon dioxide on the one hand, and to the administration of low oxygen and sodium cyanide on the other, suggests common factors of control. Acidity and state of oxidation in and about the neurone membrane concerned may be the outstanding factors controlling circulation.

The high sensitivity of the circulatory and respiratory centers to changes in oxidation agrees again with their common and coördinated function.

3323

Tissue and Blood Acidity: Coördination of the Dual Function of Hemoglobin During Suspended Ventilation.

ALRICK B. HERTZMAN AND ROBERT GESELL.

From the Department of Physiology, University of Michigan, Ann Arbor, Mich

In the paper directly following, on tissue acidity, blood acidity and pulmonary ventilation, we studied the problem of respiratory control, by regulating the shift of ions and acid molecules between the blood and tissues. A direct relation between pulmonary ventilation and blood acidity on the administration of carbon dioxide and sodium carbonate, and an inverse relation on the administration of sodium bicarbonate, were demonstrated. These diametrically opposite relationships were harmonized by referring these effects to changes in acidity of the respiratory center itself. Assuming that the acidity of the cerebro-spinal fluid reflected the changes in acidity of the respiratory center, our results agree with the theory that the acidity of the respiratory center is important in the control of pulmonary ventilation. For carbon dioxide turned the cerebro-spinal fluid acid, and increased ventilation; sodium carbonate turned it alkaline and decreased ventilation; and sodium bicarbonate turned it acid, and increased ventilation.

Changes which occur during suspended ventilation offer another approach to the study of tissue acidity, blood acidity and pulmonary ventilation, by virtue of the changes in the coördination of the dual function of hemoglobin. Changes in acidity of the arterial and venous blood of the dog, resulting from mechanical asphyxia, were studied with the continuous method of recording acidity in the circulating blood and with the hydrogen and quinhydrone electrodes. On administering over-ventilation, sufficient to produce apnea, the duration of apnea, and the relation of blood acidity to the end of apnea, were noted. Mechanical asphyxiation, with the lungs filled with either room air or oxygen, permitted partial control of blood acidity and alveolar and blood carbon dioxide pressure.

In one experiment apnea lasted 65 seconds during mechanical asphyxia, with the lungs filled with room air, and 86 seconds with the lungs filled with oxygen. The increase in acidity of arterial blood was decidedly more rapid when the lungs were filled with oxygen, and pulmonary ventilation began at a greater hydrogen ion concentration. This agrees with the common finding of an inverse relation between pulmonary ventilation and acidity of the blood.

The difference in results, and the significance of these differences with the two types of asphyxia, were considered in relation to variations in the coördination of the dual function of hemoglobin, and the acidity of the respiratory center itself. The low acidity of the arterial blood at the close of apnea, with the lungs filled with room air, was related in part to a disturbance in the coördination of the dual function of hemoglobin, resulting in a probable increase in accumulation of acid in the respiratory center. A highly reduced blood heavily laden with acid, incapable of the normal liberation of oxygen and alkali at the tissues, returns to the lungs with a small increment of carbon dioxide. Failure in oxidation at the lungs leads to retention of carbon dioxide by the blood. A highly reduced and relatively alkaline hemoglobin is in equilibrium with a low alveolar carbon dioxide tension. During mechanical asphyxia, with the lungs filled with oxygen, a highly oxidized blood lightly laden with carbon dioxide, capable of great liberation of oxygen and alkali at the tissues, returns with a large increment of carbon dioxide. Subsequent high oxidation of the hemoglobin at the lungs efficiently expels carbon dioxide. A highly oxidized acid hemoglobin is in equilibrium with a high alveolar carbon dioxide tension.

The relatively greater alveolar and arterial carbon dioxide tension at the end of apnea agrees with the common finding of the inverse relation between pulmonary ventilation and the carbon dioxide tension of the arterial blood.

A relatively high acidity and high carbon dioxide tension of the arterial blood may be indicative of a relatively high efficiency of the transport of acid from the tissues and its liberation in the lungs.

The experiments support the view of the lack of correspondence between changes in acidity in the blood and in the tissues.

The results agree with the theory that the acidity of the respiratory center is important in the control of pulmonary ventilation. They are not in disagreement with a direct effect of oxygen on pulmonary ventilation by changes in the state of oxidation of the respiratory center.

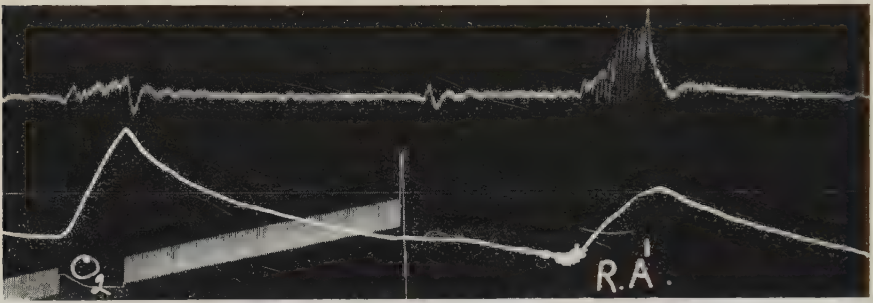

FIG. 1.

Record showing the effects of mechanical asphyxia during artificial ventilation on the acidity of the arterial blood and mean blood pressure. With the lungs filled with oxygen the increase in acidity of the blood is greater than in the second observation, in which the lungs were filled with room air. Note: the increase in mean blood pressure was considerably less.

3324

Tissue and Blood Acidity: Coördination of the Dual Function of Hemoglobin During Low Oxygen Administration.

ROBERT GESELL AND ALRICK B. HERTZMAN.

From the Department of Physiology, University of Michigan, Ann Arbor, Mich.

This paper deals primarily with effects of low alveolar oxygen on oxidation, and resulting changes in acid metabolism, coupled with the impairment of transport of acid and its elimination in the lungs.

Low oxygen mixtures (3 to 12 per cent) were administered by normal ventilation, which permits control of ventilation by the animal, and by artificial ventilation, which maintains a constant movement of air. Changes in acidity of the arterial and venous blood were studied with the continuous method, and with the quinhydrone and hydrogen electrodes.

Short administration of low oxygen (4 per cent) by normal ventilation elicited a sudden increase in alkalinity (0.1 to 0.2 pH) of the arterial and venous blood, followed by a rapid recovery on the re-administration of room air. Low oxygen administration elicited the usual increased pulmonary ventilation, which was followed by decreased ventilation on the re-administration of room air.

Similar administration of low oxygen by artificial ventilation, equal in volume to the preceding ventilation of room air, elicited markedly increased alkalinity of the arterial and venous blood. The increase in alkalinity, however, was smaller than that elicited on the administration of low oxygen by normal ventilation. The increased alkalinity of the blood resulting from administration of low oxygen may, therefore, be attributed to the washing out of carbon dioxide by excessive ventilation, and to the liberation of alkali in the process of reduction of the hemoglobin. These conclusions are supported by the recovery curves on the re-administration of room air. Whereas the normal acidity of the blood was only slowly approached on re-administration of room air with normal ventilation, with artificial ventilation it was rapidly approached. Overshooting occurred with subsequent recovery to the previous normal acid value. Such experiments indicate the accumulation of acid in the body during the administration of low oxygen, if increased ventilation is prevented.

The administration of pure oxygen, following low oxygen, led to a more rapid increase in blood acidity, and a greater suppression of respiratory movement.

Prolonged administration of low oxygen led to greater increased alkalinity of both the arterial and venous blood than short administration. This held for normal and artificial ventilation. With still longer administration, increasing alkalinity changed to increasing acidity. The directional change occurred earlier with artificial ventilation. The degree of acid change varied with the duration of administration. When markedly prolonged, the arterial and venous blood became more acid than normal. Subsequent administration of room air increased the acidity still more. Then followed a slow recovery, ending in an acid value above the preceding normal value.

With severe administration, disagreement between the manganese dioxide and hydrogen electrode occurred, indicating the appearance of reducing metabolites in the blood stream, as a consequence of marked impairment of oxidations.

The increase in pulmonary ventilation, with increasing alkalinity of the blood on the administration of low oxygen, and the decrease or stoppage of ventilation with increasing acidity of the blood associated with re-administration of room air is in agreement with the common finding of an inverse relation between blood acidity and pulmonary ventilation.

Granting an increased total acid metabolism (carbon dioxide and lactic acid) and impairment of acid transport from the tissues by a broken coördination of the dual function of hemoglobin during oxygen deficiency, and granting the reverse, a decreased total acid production and improved transport of acid on the re-administration of oxygen, the results are in agreement with a direct relation between activity and acidity of the respiratory center.

The results support the view of the importance of acidity in the control of ventilation. They do not, however, preclude the direct effects of lack of oxygen on oxidation.

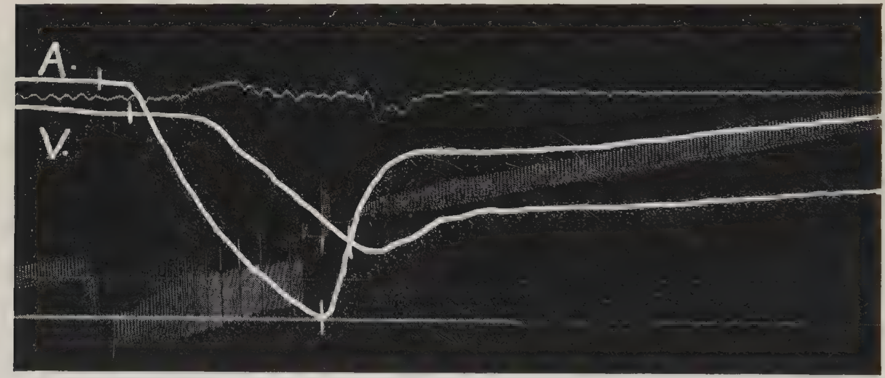

FIG. 1.

Record showing effects of administration of room air, 3 per cent oxygen, and pure oxygen on the acidity of the arterial and venous blood, on mean blood pressure and on pulmonary ventilation. The acidity curves are marked A and V, respectively. Note the cessation of respiration as the arterial blood turns acid. The recovery of the normal acidity values is incomplete at the end of the record. The blood is still turning acid in both the artery and vein.

3325

Hemorrhage and Ventilation.*

ALRICK B. HERTZMAN AND ROBERT GESELL.

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Effects of hemorrhage and reinjection on the acidity of the arterial and venous blood were studied with the manganese dioxide, quinhydrone and hydrogen electrodes. Room air was administered by normal and artificial ventilation, by the closed circuit method. Pulmonary ventilation and changes in oxygen consumption were recorded.

Hemorrhage elicited an increased alkalinity of the arterial blood, and an increased acidity of the venous blood. The increased alkalinity of the arterial blood was greater than the increased acidity of the venous blood. Subsequent injection of the blood decreased the alkalinity of the arterial blood, and the acidity of the venous blood. The extent of these changes varied with the animal and with the amount of hemorrhage. The decrease in acidity of the venous blood on reinjection may be preceded by a temporary increase in acidity. The acidity may not return to normal. On reinjection, the arterial blood may turn more acid than normal, and fail to reach the normal acid value during recovery. The acidity changes occurring during hemorrhage were accompanied by decreased oxygen consumption and increased pulmonary ventilation, and re-injection was accompanied by reverse changes.

Granting an increased oxidation of hemoglobin during hemorrhage, the increased alkalinity of the arterial blood seems related to increased pulmonary ventilation, and an increased blowing off of carbon dioxide.

Hemorrhage during constant artificial ventilation elicited similar directional changes in acidity of the arterial and venous blood. These arterial changes in acidity might conceivably be explained by the improved ventilation, due to a decreased flow of blood through the lungs. This being true, the significance of volume-flow of blood through the respiratory center is supported. The increased alkalinity of the arterial blood during hemorrhage and normal ventilation would thus be a resultant of increased ventilation of the blood through increased pulmonary ventilation, and decreased volume-flow of blood.

The results of these experiments are in agreement with the com-

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[*] With a note on an Electro-Chemical Working Hypothesis of Respiratory Control, by Robert Gesell.

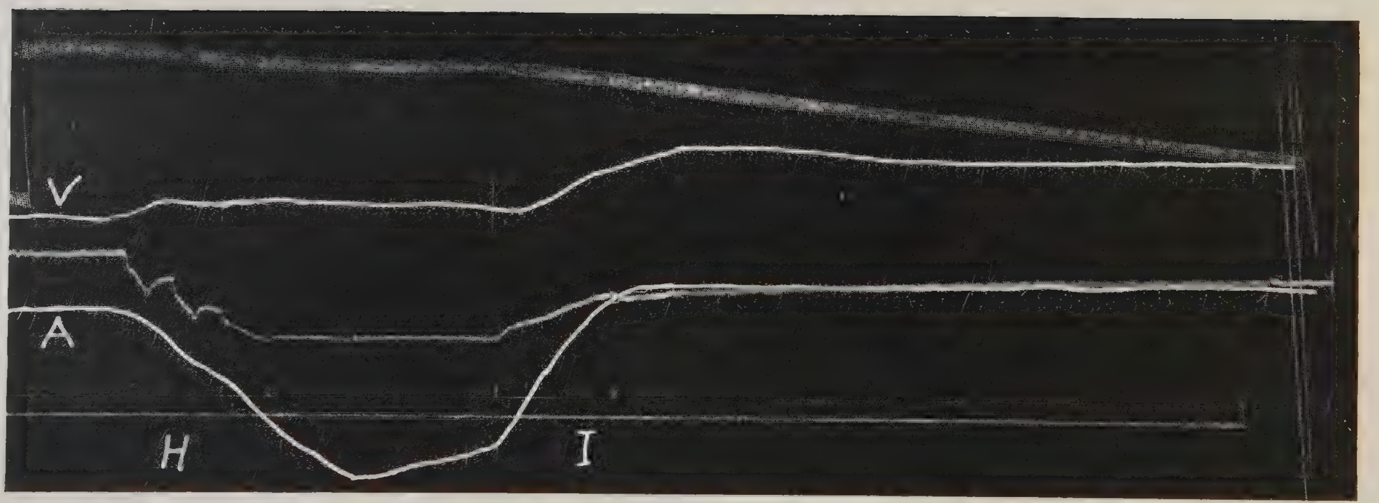

FIG. 1.

Record showing the effects of hemorrhage and reinjection on the acidity of the arterial and venous blood during normal breathing of room air. The acidity curves are marked A and V, respectively. Note the increase in pulmonary ventilation and decrease in oxygen consumption occurring with hemorrhage and the subsequent decrease in ventilation below normal on reinjection as the blood turns acid.

mon inverse relation between acidity of the arterial blood and respiratory movements. The relation between acidity of venous blood and pulmonary ventilation is more direct.

The decreased oxygen consumption during hemorrhage, and the excessive consumption following re-injection, suggest increased production and accumulation of acid in the respiratory center during hemorrhage. The failure of the venous blood to turn more acid than it does is probably related to the excessive ventilation of the arterial blood, both with respect to oxidation, and blowing off of carbon dioxide. The high oxidation of hemoglobin in the lungs should facilitate a correspondingly great reduction in the tissues.

The results here reported, as in the preceding two papers, support the view of the importance of acidity in the control of ventilation. But, similarly, they do not preclude the direct effects of lack of oxygen on oxidation.

Granting the possibility of this dual mechanism of control, the next step is to determine its probability.

If it be accepted that all biological phenomena are electrical phenomena, then all biological processes must be explained on a purely electrical basis. Whether or not the subject of respiratory control has advanced sufficiently to permit a formulation of a rational hypothesis based on electro-chemical changes might be questioned; but granting that excitation and conduction of excitation are electrical phenomena occurring with the aid of a membrane, and dependent on the composition of the fluids on each side of that membrane, an hypothesis is warranted if we can relate the behavior of the respiratory center to known changes occurring on both sides of the neurone membrane.

Heretofore, the difficulty of such approach consisted in the inability to associate consistently respiratory activity with any single variable or group of variables. It has been pointed out in preceding papers that increased pulmonary ventilation may occur in the presence of increased or decreased hydrogen ion concentration of the blood; with increased or decreased carbon dioxide tension of the blood; with increased or decreased oxygen tension of the blood. The accidental finding of the suppression of respiratory movements in volume-flow experiments by simple acceleration of the volume-flow of blood suggested the importance of a heretofore neglected factor, the metabolism of the respiratory center.

The behavior of the respiratory center on the administration of carbon dioxide and sodium carbonate was easily interpreted on the acid basis, but difficulty was encountered in harmonizing these effects with increased ventilation from intravenous injection of sodium bicarbonate. It was suggested that the "specific" action of sodium bicarbonate may be explainable on the basis of an actual increase in acidity of the interior of the cell due to the impermeability of the membrane to sodium bicarbonate and to the effect of sodium bicarbonate on the movement of acid. This was supported by *in vitro* experiments[1] and more recently by animal experiments.[2]

The increased pulmonary ventilation occurring with the administration of low oxygen, sodium cyanide, and in hemorrhage, was also contrary to the mechanism of acid control, for in all of these conditions hyperapnea is associated with increased alkalinity of the arterial blood. In these conditions the acid metabolism of the respiratory center, the coördination of the dual function of hemoglobin, and the transport of acid from the center, are factors controlling the acidity of the respiratory center. From the work of Hill[3] and Meyerhoff[4] on lactic acid, I concluded that though the amount of carbon dioxide formed as a result of decreased oxygen is diminished, this is more than balanced by an increased formation of lactic acid. Not only is the total production of acid increased within the cell, but the buffer is reduced as well. The increased ventilation could, therefore, be associated with increased acidity of the center. This increased respiration must of necessity lead to increased elimination of carbon dioxide. When oxygen is readministered the body is poor in carbon dioxide, and rich in sodium lactate, and since lactic acid is removed by returning in large part to the precursor state, avoiding oxidation, replenishment of oxygen should lead to a temporary increased alkalinity of the center. The early apnea of recovery from low oxygen is, therefore, also explainable on an acid basis.

Respiratory control seems intimately connected with changes in acidity and oxidation. A precise separation of these factors with the data at hand is exceedingly involved, for lowered oxygen transport to the center, means lowered acid transport from the center. Diminished oxygen supply to the center means not only reduced oxidations but increased acid formation as well. Beginning at the other end, increased acidity, let us say from the administration of carbon dioxide, presumably leads to lower oxidations, changes in acidity and changes in oxidation seem inseparable accompaniments, yet one of these factors might presumably be the dominating factor of respiratory control. Although I had called attention to findings of others, showing that respiration may be stimulated by the administration of carbon dioxide after low oxygen is ineffective, I was loathe to abandon the direct effects of low oxygen on oxidation.[5]

The electro-chemical hypothesis of respiratory control may assist in determining the relative effects of changes in acidity and oxidation, and is here offered as an assumption subject to test. Briefly put, the neurone membrane is a pulsatile oxidation-reduction system exhibiting rhythmic chemical and electrical changes subject to chemical and electrical influences which are determined by a combination of the metabolism of the nerve cell and by its immediate external environment.

This pulsatile mechanism has its counterpart in the behavior of the mercury peroxidate system studied by Bredig and Weinmayer,[6] and von Antropoff.[7] Pure mercury covered with a 10 per cent solution of hydrogen peroxide when brought to the proper alkalinity with sodium acetate and acetic acid exhibits a rhythmic formation and disappearance of a surface oxidation film. The behavior of the system is sensitive to changes in temperature and chemical and electrical influences resembling in many respects the behavior of biological rhythmic systems. Increased temperature, alkalinity and polarizing current may increase the rate of the rhythm and each rhythmic discharge is accompanied by a change in electrical potential. Granting Lillie's theory of conduction of excitation by self electrical stimulation, initiation as well as conduction may be accounted for by comparable electro-chemical mechanisms.

The behavior of the neurone membrane might then vary with its degree of oxidation, determined by the nature of the limiting fluids, and with the intensity of the self-generated current flowing through the membrane.

As a source of electrical energy in respiratory control I turned to the metabolism of the nerve cells and as a source of driving potential to the metabolic gradient of the nerve cell. Perhaps no living cell shows a steeper metabolic gradient rising from a level approaching zero in the neuraxone[8] to one approaching a physiological maximum in the nerve cell.[9] A high metabolism in the nerve cell proper is associated with a high production of hydrogen ions and a high rate of oxidation. Each of these factors represents a possible source of current.

The high production of acid, leading to an increased concentration of hydrogen ions within the cells leads to a polarization of the limiting membrane. Granting a greater acidity on the inside of the cell membrane the hydrogen ions concentrate at the outer surface of the membrane. The nerve cell becomes electrically positive. Current flows in the outer circuit from a region of high metabolic rate to a region of low metabolic rate and returns in the inner circuit from a region of low to a region of high metabolic rate. If this reasoning be correct, other things remaining constant, the greater the acidity of the interior of the cell the greater the acidity potential gradient due to polarization, and the greater the polarizing current.

Assuming that the cell acts as an hydrogen electrode the effects are the same. The hydrogen ion concentration of the outer fluid remaining constant, the polarizing current will vary with the hydrogen ion concentration of the cell.

The difference in rate of oxidation at corresponding parts of the neurone, represented by the oxidation-reduction gradient, is the second source of current. Comparing the nerve cell to the Voltaic cell, the region of high oxidations would correspond to the zinc plate, and the region of low oxidations to the copper plate. Current should flow from the former to the latter in the internal circuit and in the reverse direction in the external circuit.

The oxidation and acidity currents are, therefore, opposite in direction. The resultant effect of the opposing currents may now be followed during depressed oxidations from the administration of low oxygen. Decreased oxidations lead to total increased acid production with an accompanying decrease in buffer. The acidity potential gradient current is increased. Decreased oxidation reduces the oxidation potential gradient and the current set up thereby. The resultant is an increased current in the stimulating direction.

A detailed discussion of various other points is reserved for a later paper. Suffice it to say that the hypothesis at present appears to meet the known facts in various forms of respiratory stimulation resulting from the administration of carbon dioxide, sodium bicarbonate, carbon monoxide, sodium cyanide, hemorrhage, etc.

A summary of experimental data on the relative effects of acidity and oxidation indicates the greater importance of the acidity factor in respiratory control. On the other hand, the hypothesis here presented seems to meet the known facts and support a broader interpretation, giving proportionate significance to acidity and oxidation. Whatever the conclusion, metabolism of the respiratory neurone appears to be the dominant factor controlling respiration.

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3326

Glucose as a Factor in Metabolism.

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While the question of the nature and rôle of the glycuresis is still open, it has been clearly shown that glycuresis may be interpreted as a stigma imperfectness in metabolic adjustment to the quantity and quality of food intake. It is plausible that this phenomenon of the metabolic mechanism should be more noticeable in individuals with an impaired metabolism. Peculiar metabolic features of mental defectives are commonly known. We shall mention only one phenomenon because it has close bearing to the subject of the paper. While the industrial efficiency of the feeble-minded is definitely lower than that of the mentally normal, the caloric requirements of the former is hardly lower than that of the latter. The demand of the feeble minded for carbohydrates, especially breads and sweets, is striking.

Substitution of a part for bread and cereals by meat, eggs and butter (see Table I), renders them dissatisfied, unhappy and depressed, and leads to gradual and permanent emaciation (see Table II). (In addition, green vegetables with some sugar and butter in them were given *ad libitum,* approximating the caloric intake to 3000 calories.) It seemed worth while, therefore, to investigate the inter-relationship of the glycuresis and the demand for high carbohydrate intake in mental defectives.

TABLE I.

Representing a special diet (per day) with reduced carbohydrate content.

		P	F	C	
Bread	450 gm.	41	7.2	240.0	
Sugar	30 gm.	—	—	30.0	
Butter	30 gm.	—	25.5	—	
Milk	250 cc.	8.5	0.7	12.5	
2 eggs		12.0	12.0	—	
Meat	250 gm.	50.0	50.0	—	
½ Orange		0.5	—	5.0	
Total		112.0	95.4	287.5	
Calories		448.0	858.6	1150.0	2456.6

In the tables, the glycuresis is represented in mg. of sugar as estimated by Folin and Berglund's method, *i. e.,* prior to and after hydrolysis of the urine. The figures following the plus or minus signs indicate the difference after hydrolysis. The decrease noted

TABLE II.

Showing changes in body-weight on reduced carbohydrate and on customary rich carbohydrate diets. Figures in kg.

	Persons								
	1	2	3	4	5	6	7	8	9
Prior to reduced CHO diet, Dec. 25, 1925	52.7	64.8	55.9	67.5	65.7	65.0	59.8	61.8	69.5
During Jan. 12, 1926	51.8	63.9	55.4	57.5	63.2	65.2	59.5	61.8	69.1
Special, Jan. 26	50.9	62.0	53.6	55.9	61.6	64.1	58.2	61.6	69.1
Reduced, March 13, CHO diet	47.7	56.9	52.3	53.7	58.4	59.1	55.0	55.2	68.2
Total loss	5.0	7.9	3.6	13.8	7.3	5.9	4.8	6.6	1.3
After 10 days of rich CHO diet	55.0	64.3	59.3	58.0	66.1	66.3	62.7	63.6	75.0
Gain during 10 days of customary diet	7.3	7.4	7.0	4.3	7.7	7.2	7.7	8.4	6.8

TABLE III.

Illustrating the peculiarities of preformed and hydrolyzable glycuresis (in mg. of sugar per 24 hrs.) of persons with impaired metabolic mechanism (A) while on reduced CHO diet, and (B) on rich carbohydrate diet. Data for Folin's normal are reproduced for comparison.

	Persons									Day of Experiment	Normal
					(A)						
March	1	2	3	4	5	6	7	8	9		
7	504+ 93	345+157	1205+1031	468+ 44	255+ 82	295+118	558+128	267+165	245+105	4	756+ 73
8	349+276	927— 20	406+111	663+106	331+192	350+171	682+ 96	382+195	472+169	5	652+201
9	361+255	462+597	338+325	554+183	325+372	319+550	374+248	366+281	336+315	6	543+101
10	335+147	440+ 88	602+ 9	1056+ 53	331+ 90	281+262	434+ 57	417+255	296+119	7	585+ 85
11	150± 0	420+ 30	1050+193	1013+334	160+ 70		318+ 45	532+ 2	142+ 90	8	624+ 52
Average	340+154	519+158	720+334	751+144	280+161	311+275	473+115	393+179	298+160		632+102
					(B)						
April											
21	799+305	1185—219	580+536	650—176	685+111	552+278	1344+776	863+104	867+173	9	1064+910
22	628+150	1493— 14	543+334	562+187	679+622	360+180	1559—228	832+176	634+182	10	1360+771
23	1034—167	781—452	408+153	434+319	486+260	489+298	926± 0	480— 88	420+322	11	1090+200
24	583+151	771+ 3	684— 61	264+187	934— 56	756—238	764+551	828—100	842—391	12	1650+485
25	594+258	950— 90	458+362	255+165	751+460	492+252	1139—122	798+ 42	632—212	13	1170+450
Average	728+139	1036—154	555+265	433+136	707+219	530+154	1146+195	760+ 27	679+100		1267+563

TABLE IV.

Ratios $\dfrac{\text{Average glycuresis per day on customary high CHO diet}}{\text{Average glycuresis per day on reduced CHO diet}}$

Persons																			
1		2		3		4		5		6		7		8		9		Normal	
1.1	0.9	1.7	1	0.7	1.4	1.3	0.9	1.0	1.5	1.6	0.6	1.9	0.1	1.7	0.3	1.7	1.1	2.0	5.5

TABLE V.

Glycuresis, 5 to 12 P. M. urine with impaired metabolic mechanism on rich carbohydrate diet.

Date of experiment	Persons								
May	1	2	3	4	5	6	7	8	9
5	516— 54	518—84	340+20	470—29	376—20	365—39	620—179	631—128	668± 0
6	418+ 37	260+41	402+31	483—46	418+11	540—98	682— 96	531— 13	459—59
7	319— 23	194+18	423—86	319± 0	230+30	303— 6	501— 72	423+ 5	310+30
9	462—109	329—75	496—71	452—68	367—27	457—83	440—206	420— 93	437—20
Average	429— 37	325—25	415—26	431—36	348— 2	416—56	561—138	501— 58	468—12

occasionally is explained by the fact that hydrolysis apparently destroys a small part of the reducing substances.

It has been shown that in case of an efficient metabolic mechanism certain rather small deviations of the glycuresis from one day to the next occur, and the smaller the carbohydrate intake, the narrower these deviations become; on a rich carbohydrate diet the glycuresis increases generally, but the largest part of this increase consists of post-hydrolysis products. This is illustrated by a set of data reproduced from Folin's observation on a normal person (see Table III).

Estimations of glycuresis on our patients were made during 4½ months of observation, almost daily. Table III represents the data only for 5 consecutive days of dieting, and 5 days during customary diet. The number of mg. of sugar for any person varies from day to day quite extensively, and is quite different from the average, irrespective of the diet. On the other hand, as in Folin's normal, the average of preformed glycuresis is generally decreased when the carbohydrate intake is reduced. But the post-hydrolysis glycuresis is relatively, and even absolutely in the majority of the cases, increased in quantity with diminished carbohydrate intake. It can be seen by comparing the averages for any patient during the high and reduced carbohydrate diets. This condition is represented still more clearly by the ratios between averages, calculated on the basis of all the data available (see Table IV).

The glycuresis of normals, on a rich carbohydrate diet, consist of 5.5 times as many complex products (post-hydrolysis figures) as were present on a reduced diet, while the preformed glycuresis (ante-hydrolysis figures) was increased only twice. For defectives, however, the reverse is found; the increase was made up mostly by the preformed glycuresis, and not the complex metabolites. Moreover, persons 1, 2, 4, 6, 7, 8, showed a decreased post-hydrolysis glycuresis on a high carbohydrate diet, which is shown by ratios being below 1.

We conclude that a reduced relative quantity of carbohydrate in the diet of individuals with impaired metabolism renders their metabolic mechanism inefficient. The tendency to reduce preformed glycuresis exists, as is shown by reduced preformed glycuresis, but it lacks due steadiness and regularity. The ability of assimilation of metabolites, shown as post-hydrolysis glycuresis, is decidedly diminished, and the loss of body weight fulfills the picture of inefficiency of the metabolism on such a diet, in spite of the fact that the caloric value of it corresponds to the theoretical standards.

Hence the impaired metabolic mechanism of defectives evidently requires a larger relative amount of carbohydrates in their food. Within the organism carbohydrates circulate in the form of glucose. Therefore, our inference is that the circulating glucose is a factor in the metabolic mechanism and its effect increases with the increase of its quantity.

There is probably an increase in the quantity of circulating glucose in our patients, when after their last meal they rest comfortably in the living room. Apparently during this period glucose is not used as a source for muscular energy and is available as a factor in metabolism. Therefore, we ought to expect that in the urine collected from 5:00 P. M. there will be decreased quantity of the hydrolyzable products. Table V records the glycuresis in the urine collected from 5:00 P. M. to midnight. There is none or very little of post-hydrolysis reducing substances, which is in full conformity with the idea that glucose is a factor in metabolism.

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Protective Power of Serum of Animals Fed Pneumococci or Tissue of Animals Killed by Pneumococcus.

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In an earlier paper on the immunization of rats to pneumococcus, produced by feeding the tissues of animals killed by the same organism, brief mention was made of the fact that the blood of the immune animals contains protective antibodies.[1] The present article is a report of the experiments made with sera of rats and dogs. The rats were fed either the infected tissue, the living pneumococcus, or the acid-killed germ. Such animals have already been shown to possess immunity to intraperitoneal injection of virulent pneumococci.[2, 3] The dogs were fed either the living pneumococcus, or infected rabbit tissue. The object was to learn whether the serum

TABLE I.

Protective power of serum of rats immunized against pneumococcus, by feeding tissues of animals killed by the same organism. Mice were used in the tests. S = Survived. D = Died -days.

	Nov. 11/25			Nov. 19/25			Nov. 21/25			Nov. 23/25			Nov. 25/25			Dec. 4/25			Feb. 24/26		
Dose cc.	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 186	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 187	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 189	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 191	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 199	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 192	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 275
10^{-8}	S			S																	
10^{-7}	D2			D2			D3 D2			D3 S			D2 D2			D2 D2			D3		
10^{-6}	D2	D2	S	D2	D2	S	D2 D2		D2	D2 D2	D2	D10	D2 D2	D2	S	D2 D2	D2	D2	D2	D3	D3
10^{-5}	D2	D2	S	D2	D2	S			S		D3	S		S	S		D2	D2		D2	D2
10^{-4}		D2	S		D2	D2			D2 S		D2	D9		D2	S		D2	D2		D2	D4
10^{-3}		D2	S		D2	S			D3 S		D2	D3		D2	D2		D1	D2		D3	D2
10^{-2}		D2	S		D2	D3			D2 D3		D2	D2		D2	D2		D1	D1		D2	D2
10^{-1}						D2															

of such animals would protect mice against an intraperitoneal injection of virulent pneumococci. All experiments were done with type I. The preparation of the infected tissue, of the pneumococci, and their feeding, have been described elsewhere.[1, 2, 3]

To test an immunized rat's serum, the neck was shaved, the rat made unconscious by a blow across the head, the jugular vein cut, and the blood collected and centrifuged. A group of mice would be injected intraperitoneally, with graded doses of a 24-hour culture

TABLE II.
Protective Power of Serum of Dogs Fed Tissues of Rabbits Killed by Pneumococcus. S=Survived. D=Died -days.

	Feb. 2/26			Feb. 11/26			Feb. 15/26			Feb. 20/26		
Dose cc.	Pn. Alone	Pn. + Serum of Control Dog No. 6	Pn. + Serum of Treated Dog No. 1	Pn. Alone	Pn. + Serum of Control Dog No. 5	Pn. + Serum of Treated Dog No. 2	Pn. Alone	Pn. + Serum of Control Dog No. 7	Pn. + Serum of Treated Dog No. 3	Pn. Alone	Pn. + Serum of Control Dog No. 8	Pn. + Serum of Treated Dog No. 4
10^{-7}	D2			D3			S			D2		
10^{-6}		S	S	D1	D3	S	D2	S	S	D2	S	S
10^{-5}		D2	S		D3	S		D4	S		D4	S
10^{-4}		D2	S		D3	S		D3	D7		D6	S
10^{-3}		D2	D3		D3	D3		D3	D5		D2	D5
10^{-2}		D2	D2		D2	D2		D4	D2		D2	D3

TABLE III.
The protective power of the sera of Dogs 5, 6, 7, and 8 after they were fed live pneumococci. S=Survived. D=Died -days. The figure preceding D or S gives the weight of the mouse tested.

	Apr. 9/26				June 15/26			
Dose cc.	Pn. Alone	Pn. + Serum of Control Dog No. 10	Pn. + Serum of Treated Dog No. 5	Pn. + Serum of Treated Dog No. 6	Pn. Alone	Pn. + Serum of Control Dog No. 11	Pn. + Serum of Treated Dog No. 7	Pn. + Serum of Treated Dog No. 8
10^{-9}					22 S			
10^{-8}	S				22 S			
10^{-7}	D3 D3				24 D2	23 S	23 S	20 S
10^{-6}	D3 D3	S	S	S	25 D2	21 S	20 S	21 S
10^{-5}		S	S	S		21 D2	22 S	21 S
10^{-4}		D3	S	S		22 D3	22 S	21 D3
10^{-3}		D2	D4	D5		22 D2	23 D2	22 D2
10^{-2}		D2	D6	D3		21 D2	20 S	21 D3

TABLE IV.

Comparison of protective power of serum of rats immunized by feeding pneumococci (HCl killed or living) and by subcutaneous injection of dead bacteria. S = Survived. D = Died -days. The figure preceding D or S gives the weight of the mouse tested.

	August 30, 1926.					September 8, 1926.				
Dose cc.	Pn. Alone	Pn. + Control Serum	Pn. + Serum of Rat Immun. by feeding live Pn.	Pn. + Serum of Rat Immun. by feeding HCl killed Pn.	Pn. + Serum of Rat Immun. by Subcut. Inject.	Pn. Alone	Pn. + Control Serum	Pn. + Serum of Rat Immun. by feeding live Pn.	Pn. + Serum of Rat Immun. by feeding HCl killed Pn.	Pn. + Serum of Rat Immun. by Subcut. Inject.
10^{-9}	18 D2					18 S				
10^{-8}	22 D2					21 S	19 S	19 S	20 S	20 S
10^{-7}	24 D2	19 S	20 D2	21 S	21 S	21 S	21 S	21 S	21 S	21 S
10^{-6}	26 D2	22 D2	23 D2	23 S	23 S	23 D1	22 S	22 S	23 S	23 S
10^{-5}	28 D2	25 D2	26 D2	26 S	26 D2	26 D2	24 D2	24 S	25 S	25 D5
10^{-4}		26 D3	27 D2	27 D2	27 D2		26 D2	26 D2		27 D2
10^{-3}		28 D2	28 D2	28 D2	32 D2					
10^{-2}			32 D2	30 D2	38 D2					
	September 14, 1926.					September 21, 1926.				
10^{-9}	19 S					18 D3				
10^{-8}	21 S					20 D2				
10^{-7}	23 D2	19 D2	21 D2	21 D2	21 S	24 D2	18 D1	18 D2	18 D2	19 S
10^{-6}	24 D2	22 D2	22 D2	22 D2	23 S	25 D2	20 D2	21 D2	21 D2	24 S
10^{-5}	25 D2	23 D2	23 D2	23 D2	23 S	27 D2	25 D2	25 D2	25 D2	25 D3
10^{-4}		24 D2	25 D3	25 D2	25 D2		25 D2	26 D2	26 D2	26 D2
10^{-3}		23 D2	26 D2	26 D2	25 D2		27 D2	27 D2	27 D2	28 D2
10^{-2}			27 D2	30 D2	31 D2			29 D2	29 D2	32 D1
	October 1, 1926.					October 7, 1926				
10^{-9}	19 S					16 S				
10^{-8}	20 S					18 D2				
10^{-7}	20 D2	19 D2	19 D2	19 S	19 S	19 D2	16 D2	16 S	17 D2	18 D2
10^{-6}	22 D2	20 D3	20 D2	20 S	20 S	20 D2	19 D3	19 D2	19 D2	19 D3
10^{-5}	24 D2	21 D2	21 D2	21 D2	22 S	20 S	19 D2	19 D2	19 D3	19 D3
10^{-4}		22 D2	22 D2	23 D2	24 S		20 D2	20 D3	20 D2	20 D3
10^{-3}		26 D3	27 D2	27 D2	27 S		20 D2	20 D3	21 D2	21 D2
10^{-2}			27 D2	27 D2	28 S			21 D2	22 D2	22 D1
	November 10, 1926.									
10^{-9}	17 S									
10^{-8}	20 D3									
10^{-7}	20 D3	19 D2		18 D2						
10^{-6}	22 D2	20 D2		20 D2						
10^{-5}		21 D3		21 D2						
10^{-4}		22 D2		23 D2						
10^{-3}		23 D2		23 D2						
10^{-2}				23 D2						

of pneumococcus, (in a volume of 0.20 cc.) plus 0.20 cc. undiluted serum. Another group of mice would be injected similarly with pneumococcus culture and control rat serum. An additional small group was used to determine the minimum fatal dose of the culture employed. In the dog experiments, some animals were fed the tissues of rabbits killed by pneumococcus, others were fed the living pneumococci. Following the feeding of 275 gm. of tissue, or of the germs from 750 cc. culture per day per dog, for about 2 weeks, the serum was tested in the same manner as the rat serum. There were 4 dogs which were fed the infected rabbit tissues, and 4 controls. These 4 controls were subsequently fed living pneumococci, and compared with 4 new controls, thus making 8 control and 8 experimental dogs. Tests were made for the presence of agglutinins and precipitins, in the sera of the 4 dogs which were fed the infected tissue. Neither agglutinins nor precipitins appear to be present.

Table I shows that the sera of rats, fed tissues of animals killed by pneumococcus possess varying degrees of protective power. The serum of rat 186 protected against 0.01 cc., which was 100,000 times the fatal dose for control mice. Sera of rats 192 and 275, on the other hand, showed no protective action. The others, 187, 189, 191, and 199, were intermediate between these extremes, and uniformly protected against larger doses, than did normal control serum. The sera of immunized rats 189, 191, and 199 were examined for agglutinins and precipitins. The abdominal washings of a mouse killed by pneumococcus injection were used. Neither agglutinins nor precipitins were found.

Table II shows that the sera of dogs fed the tissues of rabbits, killed by injecting pneumococcus, possess protective antibodies in varying amounts. The controls numbered 5, 6, 7, and 8, were subsequently fed live pneumococci and retested. The results are given in Table III. The sera of Dogs 5, 6, 7, and 8 all show an increase in protective power, following the feeding of the tissues of rabbits killed by pneumococcus.

The sera of 3 adult rabbits were examined before and after feeding the living pneumococci from 500 cc. growth per day for 2 weeks. No protective substances were found in their sera.

Following this, experiments were begun to learn whether rats, when immunized by subcutaneous injection of heat-killed pneumococcus, (60° C., ½ hr.) produced protective antibodies with greater regularity than rats immunized by feeding. For this purpose 3 groups of rats were prepared. Each rat in the first group was fed the living pneumococci from 50 cc. culture per day for 13 days (June 30 to July 23). Each of the second group was fed the acid-killed germs from the same amount of culture for 16 days (June 30 to July 23). Each of the last group received subcutaneously 0.10 cc., 0.20 cc., 0.40 cc., and 0.50 cc. of undiluted heat-killed culture at weekly intervals (on July 2, 9, 17 and 23rd). At different times an animal from each group was sacrificed, its blood centrifuged, and its serum tested for protective substances in the manner described above. Table IV gives the results.

An examination of Table IV shows that there is considerable variation in the quantity of protective antibodies in the sera of rats immunized in a given manner, whether it be by feeding or by subcutaneous injection. It is perhaps possible that the presence of such protective substances is transitory or that they appear in the blood of different animals at different intervals of time following immunization, making detection difficult. Or the findings may be interpreted as further evidence that immunity need not be accompanied by the presence of humoral antibodies.

The results of the rat and dog experiments suggest that a larger animal may be found which, when fed pneumococcus cultures, will produce a serum of therapeutic value. We are now making experiments with this object in view.

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[2] Ross, Victor, *J. Immunol.*, 1926, xii, 237-249.

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Some Factors Governing Renal Function.

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With the ultimate objective of finding a satisfactory method for the quantitative demonstration of impairment of renal function in humans, the authors have studied the rate of elimination of urea, as compared with the amount of urea in the blood and with the simultaneous excretion of water. The previous work of Ambard,[1] McLean,[2] and Addis,[3] and of the associates and critics of these experimenters, has been considered in detail.

In regard to the relationship of blood urea levels to rate of urea excretion, it will be recalled that there are two divergent schools of thought. The older group, of which Ambard and McLean are the outstanding examples, have shown that the rate of excretion is a parabolic function of the concentration of urea in the blood, according to the equation $Ur = K\sqrt{D}$. $Ur =$ Gm. urea per liter blood. $D =$ Gm. urea per 24 hr. (Measured over a short period and extrapolated for the 24 hours.) Addis, and later Adolph,[4] on the other hand, under conditions involving a large provocative ingestion of either urea of water, or both, have demonstrated a linear relationship, $Ur = KD$. Under ordinary conditions, however, the relationship approaches, but does not equal, the parabolic relationship proposed by Ambard.

By combining our own data with the normal cases reported by McLean and by Addis (excluding the cases where provocative doses had been given) we were able to calculate this relationship in 220 normal individuals. By simultaneous determination of the Pearson correlation coefficient, the correlation ratios and comparison of these with each other and with the probable error, according to the method of Blakeman,[5] it was possible to demonstrate that the relationship in these normal cases was not linear. By taking the averages of all values of D for each value of Ur and plotting them logarithmically against the values of Ur, a curve is obtained, the equation of which is $Ur = .0340(D)^{.72379}$. This defines a sub-parabola, lying intermediate between the parabola of Ambard and the line of Addis.

Over the range of the values of blood urea usually found in normals and in nephritics (exclusive of the late stages of uremia) this relationship varies from that proposed by Ambard (taking a constant of 0.08) by less than the ordinary variations of either. Assuming a constant of 0.10 in the Ambard equation defines a limit dividing the values found in normals, or incipient nephritics, from those found in the more advanced stages of the disease. This curve includes 96.5 per cent of the normal cases. A similar limiting curve drawn on a basis of the Ambard equation, with a constant of 0.075, gives us a basis of division between the nephritics of all grades and the normal, and includes 96 per cent of all the nephritic cases studied. The area intermediate to these two curves is an indeterminate area, with a suspicion of pathology.

To simplify calculation, we have altered the original Ambard formula by inverting it and adding a coefficient 7.5, which permits us to group our subjects on the scale of 100, in accordance with the McLean convention. This final form of the Ambard expression is $7.5\sqrt{D}/Ur = F$—functional capacity of kidney on scale of 100. The following table gives a summary of the distribution of our cases on this basis:

	Normal Cases	Cases with Renal Impairment
F above 100 (high normal group)	76%	4%
F between 100 and 75 (low normal or doubtful group)	20.5%	56%
F below 75 (deficient group)	3.5%	40%

It will be patent to the observer that this formula is a simplified form of the well-known McLean index, with the factors for body weight and urine concentration omitted. These two factors, and especially the latter, we have found not only to add nothing to the validity of the determination, but in many cases to lead to false results, especially in normals. The urine concentration factor in the McLean index is based upon the second law of Ambard, which may be expressed $D/D' = \sqrt{C'}/\sqrt{C}$ when Ur is constant and C is the grams urea per liter urine. This relationship has been investigated by Addis and by us, and found to be entirely unreliable. In repeated investigations upon the same individual, where Ur was the same, the two ratios differed from each other by values up to 360 per cent.

This leads to the consideration of the nature of the relationship between variations in urine volume and urea output. Taking the same 220 cases, from which were derived the blood urea-output relationship, together with the averages of output for each value of urine volume, it is found that up to the rate of volume output of 100 cc. per hour the urea output increases as a linear function of the volume output, the equation being roughly $D = V/2$ where $V =$ cc. urine per hour. Above this limit the relationship becomes obscured by other factors. This "augmentation limit" phenomenon has been previously demonstrated by Austin, Stillman and Van Slyke,[6] and confirmed by Rabinowitch.[7]

The most reasonable explanation of this partial correlation is, we believe, that proposed by Crawford and McKintosh.[8] When there is no excess of water to be eliminated, the elimination of water is determined by the amount of solutes to be excreted, a condition in which urea would be a major factor. Hence for each blood urea level there would exist a definite volume for the excretion of urea. In the presence of excess water, however, there would be an elimination of water regardless of the amount of urea simultaneously excreted, and there would be no quantitative relationship.

In case this is the true state of affairs, it becomes evident that the inclusion of a volume correction in the ratio for the expression of functional capacity is unnecessary and inadvisable, since, statistically speaking there is a determined volume for the excretion of urea at each blood urea level. In individual cases there is wide variation from this statistical mean, but these variations have not been shown to be functionally diagnostic.

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3329

Galvanotropism and "Reversal of Inhibition" by Strychnine.

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Definite neuromuscular effects are produced in a variety of organisms by the passage of a constant electric current. The character of the induced galvanotropic curvatures and movements has played a certain part in the development of the tropism doctrine (*cf.* Loeb, 1918), but these reactions still await quantitation, which is difficult, as well as fuller utilization for the analysis of animal movements. The interpretation of galvanotropism in metazoans turns upon the at present necessary and sufficient assumption that the current serves to excite definite groups of nerve cell bodies (presumably as determined by the axial orientation of these cells with respect to the polarity of the current.[1, 2, 3] The effects are such that among annelids, for example, the animal typically extends and lengthens when the head is toward the cathode, but shortens when the current is in reverse direction. These effects are in certain respects similar to those produced by neurophil drugs. Strychnine, for example, induces a similar and comparable elongation, whereas nicotine leads to shortening. It was considered that if the effects of such alkaloids are indeed due to selective or differential unions with particular groups of nervous elements, the result of combining the action of a substance of this type with that of the electric current should be merely an accentuation of the primary action, since the response induced involves activity of the same nerve muscle groups. In certain cases this turns out to be true.

The effect of strychnine is of especial interest. In the spinal cord of vertebrates it is usually supposed that the strychnine effect is due to abolition of the inhibitory component of normal coördination, so that the inhibitory effect is transposed into an excitatory one.

The cathodically galvanotropic orientation of nemerteans, *Lineus*, and the anodic orientation of the gephyrean *Echiurus*, are reversed by the action of strychnine under conditions such that the typical "reversal of inhibition," or reversal of reaction to tactile excitation, induced by this substance is apparent. Nicotine does not give this result. Since it is necessary to assume that the strychnine effect is due to action upon the central ganglia, and since the galvanotropic effect is supposed to depend upon action of the current on nerve cell bodies of the central ganglia, it must be assumed that the locus of reversal by strychnine is not perikaryial, but presumably synaptic.

3330

An Experimental Study of Mauthner's Cell in Amblystoma.*

S. R. DETWILER.

From the Zoölogical Laboratory, Harvard University.

Anatomical descriptions of the connections of the two giant Mauthner neurones in the central nervous system of teleost fishes[1, 2] and amphibia[3] have strongly suggested a functional rôle in the equilibration of the organism during the performance of rapid and intricate movements.

The two perikarya lie in the medulla, at the level of entrance of the acoustic nerve roots. The axones decussate in the ventral commissure and course caudally through the spinal cord where synaptic connections are made with the motor horn cells. In addition to establishing a direct connection with the VIII root fibers, many additional dendritic connections have been described.[2, 3]

The nature of the synapse of this cell has been investigated by Bartelmez[2] and by Marui.[4] The latter author has also described certain effects of over-activity upon the morphological structure of the synapse.[5]

The developmental specificity of this cell became apparent during experiments in which the cephalic end of the spinal cord was replaced by an extraneous medulla. In some animals with two medullas in series, four Mauthner's cells with axones coursing through the cord have been noted.[6, 7]

The experiments briefly reported here deal with the elimination of one of the two neurones normally present, with a view to studying the effect of such absence upon the activity of the organism. One ear vesicle of embryos in the tail-bud stage of development was excised, thus exposing the medulla. A very small piece of the lateral wall of the medulla was then excised, after which an otic vesicle from another embryo was grafted (with normal orientation) into the excavated ear region. For comparison, other experiments were carried out in which the ear vesicle alone was removed. The behavior of larvae lacking one ear was found to be essentially similar to that described by Greene and Laurens.[8] The animals in which an attempt has been made to remove Mauthner's cell (26 cases), fell decidedly into two groups as regards behavior. In one group (group *A*) equilibratory disturbances were either slight or entirely absent but the animals exhibited 'jerky' swimming, they were rapidly exhausted and were unable to execute the normal sustained rapid swimming reflexes. In the other group (group *B*) the functional disturbances were essentially like those lacking an ear vesicle (bend of head towards operated side, body torsion, marked flexion of fore limb on operated side, and extension of opposite limb, emprosthotonus, 'corkscrew' swimming, coming to rest in almost any position, general inactivity and in some cases rapid exhaustion).

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* The effects of embryonic ear extirpations upon development of Mauthner's cell in Amblystoma have been studied by Doctor W. F. Greene in the Yale Zoölogical Laboratory. His results have not yet been published.

An examination of sections showed that in five cases belonging to behavior group *A,* Mauthner's fiber was absent, but that the ear and its nervous mechanism were normally developed. In behavior group *B,* regardless of whether Mauthner's fiber was present or absent, defective development of the ear and its nervous connections were observed. A correlative study of the structural and functional abnormalities for the groups as a whole shows that in the absence of one Mauthner's cell essentially normal posture is maintained. This suggests that unless other tracts are compensating for its loss, Mauthner's cell is not primarily bound up with the equilibratory reflexes. The type of response elicited by the lack of Mauthner's fiber, suggests that this giant neurone plays an important rôle in transmitting impulses which are concerned in sustained coördinated rapid reflexes.

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3331

The Relation Between Pigmentation and Injury in the Conjunctiva of the Frog Tadpole.

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From the Biological Laboratory, Clark University.

It has been shown[1] that following the removal or extensive injury of the conjunctiva of frog tadpoles *(Rana catesbeiana* and *R. clamitans)* the regenerated conjunctiva becomes heavily pigmented by epidermal melanophores, and that the pigmentation persists for several months.

Experiments have been made to discover relationships that might exist between the extent of injury and the amount of pigmentation, as well as between the locus of injury and distribution of the pigment. The conjunctivas of tadpoles of *R. catesbeiana,* 1 and 2 years old, have been injured by incisions approximately 1, 2 and 3 mm. long, in different places. In some cases small pieces of the conjunctiva were cut out entirely. The results were as follows: When incisions were made in places about equally distant from the edge of the conjunctiva, the resulting pigmentation was proportional to the length of the cuts. In the case of the minimum injuries, the distance from the surrounding pigmented skin was a determining factor. The minimum wound that caused pigmentation was an incision about 1 mm. long, located not more than 1 mm. from the edge of the conjunctiva. When cuts 2 or 3 mm. long were made near the center of the conjunctiva pigmentation appeared, but not if the cuts were less than 2 mm. long. Small cuts or wounds near the edge of the conjunctiva were followed by pigmentation only of the tissues surrounding the wounds and between the latter and the edge of the conjunctiva. Melanophores were never seen on the side of the small cuts proximal to the center of the conjunctiva. The pigmentation thus produced by injury persisted for 4 months, when the experiments were discontinued. Within a few days after the injury the melanophores lost their typical spindle shape with dendritic processes, and became rounded contracted cells. This change in shape appeared to be independent of the state of the melanophores in the rest of the body, and led to the idea that the contraction was due to some local cause, different from that which controlled the skin melanophores.

It was concluded that the amount of pigmentation by epidermal melanophores in the regenerating conjunctiva is directly proportional to the extent of injury, and inversely proportional to the distance of the wound from the edge of the conjunctiva; that in tadpoles which are just approaching metamorphosis, the pigment cells persist for at least 4 months; and that the initial expanded phase of the melanophores is followed by contraction, which appears to be permanent.

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SCIENTIFIC PROCEEDINGS.

Western New York Branch.

Clifton Springs Sanitarium, December 11, 1926.

3332

The Inactivation of Crystallized Urease by Water and Its Prevention.

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Crystallized urease[1, 2] diluted with 100,000 times its weight of water, so that each cubic centimeter contains approximately one unit, is very rapidly inactivated at room temperature. The curve obtained when time is plotted against activity is practically a rectangular hyperbola. This inactivation by water is prevented more or less completely by amino acids, proteins, buffered cyanide, phosphates, and by many colloids other than proteins. Some of these colloids are: gum arabic, boiled starch, glycogen, aluminum hydroxide gel, gum mastic suspension and castor oil suspension. Glycocoll and alanin in 1 per cent solution do not afford complete protection, but neutral 2 per cent gum arabic protects perfectly. On account of this fact, 2 per cent gum arabic can be used to dilute crystallized urease when it is necessary to do so in order to test its activity. The protective action of phosphates is greater at pH 6.1 than at neutrality. Urease that has been largely inactivated by water can be partly reactivated by adding either gum arabic or amino acids. Urease is doubtless protected by urea, and also appears to protect itself. Urease crystals dissolved in only 1300 times their weight of water lose no more than 2 or 3 per cent of their activity during the first hour, as compared with a urease solution made by diluting the same quantity of crystals to the same volume, with 2 per cent gum arabic solution.

Impure urease solutions are largely protected by their impurities and show no measurable protective action by added amino acids and colloids when one unit of enzyme per cc. is tested for a period of 5 minutes. However, when diluted to contain only 0.1 unit per cc. and allowed to react with urea-phosphate for as long as 50 minutes at 20° C., impure urease plainly shows the protective action of amino acids and of colloids.

Rockwood[3, 4] and Rockwood and Husa[5] have maintained that the effect of amino acids on impure urease is largely a promoter instead of a protector one. Sherman and Walker[6, 7, 8] have disputed this, and claim that the effect is a protective one. My work with crystallized urease fails so far to show any promoter action, since the addition of amino acids or of colloids has never caused diluted urease to decompose urea more rapidly than an equal amount of undiluted urease.

The facts described in this paper show why urease is destroyed by dialysis. It is possible that many enzymes besides urease are capable of inactivation by water. The ability of urease to protect itself, when it has been not too greatly diluted, may be due to a combination of the electro-positive and electro-negative groups in one urease molecule with the electro-negative and electro-positive groups in another urease molecule. Amino acids possibly act in much the same manner, uniting with urease with both their amino and carboxyl groups. Colloids probably protect urease by condensing it upon their surfaces and thus preventing its dispersion.

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3333

Changes in the Growth of Skeletal Muscle Following Thyroidectomy in the Sheep.

ETHEL D. SIMPSON. (Introduced by H. S. Liddell.)

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A comparative study of skeletal muscle from 5 normal sheep and 11 thyroidectomized animals was undertaken, to determine whether definite structural changes in the fibers are present which would account for the development of the pot-belly and general muscular weakness in cretins. Following early thyroidectomy, the normal development of cytoplasm is prevented, and the ratio of muscle to sarcoplasm in the cretin remains more nearly like that in the young animal, than that in the normal of the same age. No degeneration of the fibers is evident, and all the structures of normal muscle are demonstrable.

3334

Auditory Conditioned Reflexes in the Thyroidectomized Sheep and Goat.

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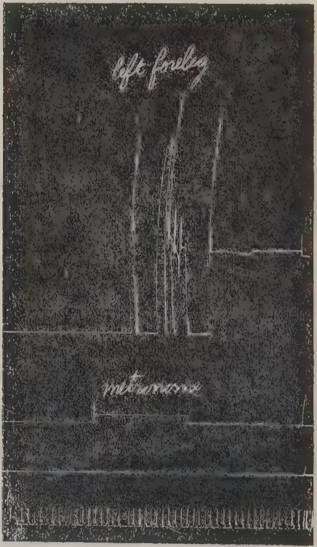
The lethargy of the cretin sheep or goat, and its apparent indifference to its environment, suggest a fundamental defect in cerebral function, but when the animal is subjected to simple learning tests the deviation from the normal, under experimental conditions, is less striking. For example, the animal, unless it is suffering one of its recurrent periods of prostration, learns the maze with a single *cul de sac* as well as the control. It is only when the blind alley is reversed in position, at every trial, that striking deviations from normal behavior appear. The extreme complexity of the animal's response in the labyrinth makes the analysis of the data in physiological terms very difficult. For this reason the maze studies have been abandoned in favor of the conditioned motor reflex method.[1]

A systematic investigation of cerebral activity involves, according to Pavlov,[2] two problems, a study of the analyzers (receptors with their central terminations) and of the mechanism for the formation of temporary nervous connections. In a preliminary study[3] it was demonstrated that the formation of a simple tactile conditioned reflex was effected as easily in the cretin as in the normal sheep. Secondary conditioned reflexes of proprioceptive origin added to the primary tactile reflex were observed in both operated and control animals. Since the alternating maze problem exhibited marked differences between cretin and normal, which were shown neither in the simple maze nor in the formation of the simple conditioned reflex, it was decided to attempt to form more difficult conditioned reflexes, viz., discriminative and delayed reflexes to the sound of the metronome. It was necessary at first, however, to discover whether or not the auditory analyzer in the cretin could be employed in building up simple conditioned reflexes.

Two cretin sheep and a cretin goat were trained to respond by a movement of the left foreleg to the sound of the metronome by combining the conditioned auditory stimulus with an electric shock (unconditioned stimulus) applied to the shaved skin of the foreleg. An auditory reflex was easily formed in all three thyroidectomized animals, and the figure illustrates the conditioned response of one of the cretin sheep to the metronome at 60 beats per minute. The time record is in seconds.

FIG. 1.



Results so far obtained with the conditioned reflex method indicate that in the thyroidectomized animal three analyzers (tactile, proprioceptive, and auditory) may be employed in the formation of temporary nervous connections, and that these connections are readily formed, when the reflex to be established is of the simple or simultaneous type. Having demonstrated the possibility of forming simple auditory reflexes in the cretin sheep and goat, we are now attempting to establish the more difficult discriminative and delayed conditioned reflexes.

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Southern Branch.

Tulane University, New Orleans, La., December 2, 1926.

3335

Electrographic Studies of Conductivity in Cardiac Muscle.

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This abstract presents the results of studies with the string galvanometer which confirm and extend previous observations[1] upon the recovery of conductivity, and the effect of the blocked impulse upon subsequent conduction in the quiescent, excised, atropinized turtle heart. The previous work was with the kymograph.

The observations were incidental to experiments directed toward other ends. They are based upon studies of approximately 30 turtle hearts, from some 15 of which simultaneous myograms and electrograms were obtained. These demonstrate that although the electrical and mechanical conduction intervals may not appear identical, the curves illustrating the recovery of conductivity are parallel. The electrical are more accurate than the mechanical.

The results fall into three divisions. (1) Curves illustrating the recovery of conductivity between auricle and ventricle, with and without compression at the A-V groove. These are similar to those already published,[1] excepting that in some the deviation of the individual points from a smooth curve is less pronounced. They demonstrate that not only are conduction times for all rest intervals increased by compression, but also that the curve returns much less promptly to a resting level. A recovery curve for the human heart was reported by Ashman and Herrmann.[2] (2) Confirmation of the fact that a very premature blocked impulse may have no detectable influence upon the conduction time of a subsequent impulse; that a slightly later blocked impulse has a moderate effect; and that an impulse arriving at the compressed muscle so late as to just fail of transmission to the ventricle, has, on the average, 65 per cent as great an effect as a transmitted impulse. These results are identical with those previously reported. A similar phenomenon has been reported by Lewis and Master[3] for the mammalian heart. (3) Demonstration that the duration of the refractory period preceding any given auricular impulse determines the velocity with which it will be conducted, or, if blocked, its effect upon subsequent conduction. Thus, if the auricular cycle preceding that terminated by the impulse in question is short, so that the impulse is preceded by a short refractory phase, the velocity of transmission is greater than had the antecedent cycle been long. This result, which is most marked with no or slight compression, confirms that found by Lewis and Master for the mammalian heart. If the cycle antecedent to an auricular cycle terminated by a blocked impulse is short, the effect of the blocked impulse upon subsequent conduction is greater than had the antecedent cycle been long.

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3336

Factors in the Metabolism of Galactose.

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From the Department of Bio-Chemistry of the School of Medicine, Tulane University, New Orleans, Louisiana.

While there are as yet no satisfactory chemical methods available for the rapid determination of galactose in blood, the resistance of this sugar to fermentation by yeast offers a means for the approximate estimation of the amount of this substance present in the blood following its administration. As in the determination of pentoses, previously described,[1, 2] it has been assumed that the difference between the total non-fermented reduction of the blood and the usual rather constant residual reduction gives a measure of the amount of galactose present.

The details of this series of experiments may be summarized as follows: The sugar (or sugars) in aqueous solution was injected into the marginal ear vein of a fasting, medium sized (about 2 kilos), male rabbit. Blood samples were taken before, and at hourly intervals afterwards. The blood was analyzed for total reduction and reducing power after fermentation.[1, 2]

In view of the facts that the tolerance for glucose is greater after the previous administration of the same sugar, and that glucose has been reported to increase the tolerance for galactose,[3, 4] these studies have included experiments on the rate of removal of galactose from the blood stream after a previous administration of galactose, and after, or with, the administration of glucose.

The residual reduction of the blood returned to normal in 2 hours, in 2½ hours and in 3 hours, respectively, after the intravenous injection of 1, 2 and 3 grams of galactose. The previous administration of galactose or glucose, or the simultaneous administration of glucose, has not been found to have any marked effect on the rate of removal of galactose from the blood after its intravenous injection. Following the simultaneous injection of 2 units of insulin and one gram of galactose, the reducing power of the blood after fermentation was practically back to the control level in one hour. The reports of the effect of galactose on insulin intoxication have not been consistent.[5, 6, 7, 8] The results can probably be explained on a basis of differences in technique.

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3337

A Rapid Method for Typing in Pneumococcal Meningitis.

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The typing of pneumococci is a laboratory procedure which requires a considerable amount of time and for this reason its practical value is greatly minimized. Whether cultures of the organism or white mice inoculations are resorted to, time is a factor that cannot be avoided.

The choice between an agglutination reaction or a precipitation reaction to determine the specific type is rather a personal one, but the writer feels that the latter is in many ways more satisfactory and conclusive.

In pneumococcal meningitis the spinal fluid offers us the means of producing an antigen for a precipitation reaction which is most satisfactory, and permits typing of the pneumococcus present in a very short space of time.

In pneumococci infections of the meninges there are many pneumococci present in the spinal fluid. Whatever amount of fluid is available is centrifuged to throw the organisms to the bottom of the test tube. The fluid is then pipetted from the top, allowing two or three cubic centimeters to remain at the bottom. This is now shaken and we have a concentration of pneumococci much as we would have in a culture many hours old. As the pneumococcus is a bile-soluble organism, we add sodium taurocholate in the proportion of 0.2 of a cubic centimeter of a 10 per cent solution to each cubic centimeter of the spinal fluid retained. It should now be thoroughly mixed by shaking and allowed to stand for from 15 to 30 minutes to allow for the dissolution of the pneumococci. Centrifugation at high speed is again resorted to in order to clear the solution. It is then carefully pipetted off, not disturbing the sediment at the bottom of the tube. This gives us a satisfactory antigen prepared and ready for use, and not requiring an undue amount of time.

While this preparation is going on, 4 small calibre test tubes, 5 mm. x 60 mm. (ordinary capillary tubing cut in 60 mm. lengths and sealed on one end by Bunsen flame is all that is necessary) are set up in a rack, and undiluted diagnostic anti-sera for types I, II and III pneumococcus are placed in tubes 1, 2 and 3 respectively, (this sera may be obtained from the biological supply houses) and in tube 4 saline is used as a control. The amounts of sera need not be accurately measured, about 1 cm. depth in the tubes is sufficient, the amounts being approximately the same in each tube. Now an equal amount of our prepared antigen is added to each tube and mixed with the serum. A definite precipitate forms in the tube containing the specific anti-serum for the particular type of pneumococcus present. The forming of this precipitate is only the matter of a few minutes.

Setting up the reaction in tubes is not absolutely essential; a slide test may be substituted. Place 3 drops of the anti-serum for pneumococcus, type I, on a clean glass slide and add 3 drops of the prepared antigen. Mix these thoroughly and then rotate gently on the slide. Repeat this procedure using type II anti-serum, and again using type III anti-serum. A definite precipitate will be seen on the slide when the anti-serum corresponds to the particular type of pneumococcus present.

In conclusion. 1. The pneumococcus in meningitis may be typed in a satisfactory and rapid manner.

2. In many instances a positive differentiation may be quickly made between a pneumococcus and a streptococcus, avoiding the delay of tedious and time-consuming cultural studies.

Minnesota Branch.

University of Minnesota, December 15, 1926.

3338

Sodium Ricinoleate and Active Immunity Against Experimental Monkey Poliomyelitis.

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From the Division of Nervous and Mental Diseases and the Department of Bacteriology, University of Minnesota Medical School.

The experiences of one of us (Larson[1]) with the detoxifying action of sodium ricinoleate in the case of several bacteria and several bacterial toxins, suggested the possibility of actively immunizing monkeys against experimental poliomyelitis through the medium of this soap. Consequently we obtained a sample of the "M. A. strain" of poliomyelitis virus from the Rockefeller Institute of New York, through the courtesy of Dr. Simon Flexner, and established the disease in our own animals.

In general, we are proceeding according to the following plan. A virulent sample of poliomyelitis is obtained by producing the disease in an animal by the intracerebral injection of stored glycerinated virus. The animal is then killed by chloroform during the height of the disease, and immediately about a gram of the brain is removed sterilly and ground up in 5 to 10 cc. of physiologic saline solution. This emulsion is used to continue the disease in another animal and to prove the potency of the soap-virus emulsion injected into the animals being immunized. At the same time another gram of the brain is ground up and emulsified with 5 to 10 cc. of 1 per cent aqueous sodium ricinoleate. This soap-virus emulsion is injected by different routes in normal animals to establish the assumed immunity. Some time later these animals are injected intracerebrally with a virulent saline emulsion of poliomyelitis brain to test their resistance.

Approximately 60 injections of the soap-virus emulsion have been administered in the animals by the cerebral, subcutaneous or intraperitoneal route. None of these animals have developed poliomyelitis within the incubation period of the disease following such an injection, though 2 of them showed complete hemiplegia immediately after frontoparietal cerebral injection, and died within 2 or 3 days. This result is readily correlated with the destruction of brain tissue from the action of the soap itself. Injection of 0.5 cc. of soap alone into a rabbit's brain is regularly fatal, and 0.5 cc. or more of the soap is commonly fatal to the monkey by this route, but 10 cc. are easily borne when injected subcutaneously or intraperitoneally. Microscopic examination of the animals dying shortly after cerebral injection of the soap-virus mixture showed absence of the lesions of poliomyelitis in the central nervous system. It appears from these limited observations that sodium ricinoleate attenuates or detoxifies the poliomyelitis virus so that the disease is not produced in animals by injecting soap-virus mixture containing a potent virus.

Animals No. 3, 14, 15 and 17 were treated for the production of immunity and 3 normal animals (10, 19 and 21) were used as controls as follows:

Animal No. 3. Male. Javanese macaque. November 14, 1925, this animal was injected cerebrally on the left side with 0.15 cc. of filtered 5 per cent emulsion of 1 per cent castor oil soap and glycerinated brain tissue from Rockefeller Institute monkey No. 1933. The animal showed a paralysis of his right side on recovery from the anesthesia, no doubt due to the action of the soap on the brain. The control animal inoculated with the same virus in saline solution did not develop poliomyelitis, so we considered it likely that the virus was not potent in this experiment. At that time we did not realize that glycerinated virus is an attenuated virus and often does not infect the animal injected. April 17, 1926, the animal was injected in the left parietal region of the brain with 0.7 cc. of fresh soap-virus emulsion from a paralyzed monkey which was killed the same day at the height of an attack of the disease. The control animal, injected with this material at the same time, developed poliomyelitis in 5 days, and was killed on the 6th day. Microscopic examination confirmed the presence of the disease. We considered that immunity had possibly been induced at this time in animal No. 3.

Animal No. 14. Female. *Macacus rhesus.* May 25, 1926, fresh soap-virus emulsion, prepared from a poliomyelitis animal which had been killed immediately previously, was injected into the brain of this animal. The monkey showed a hemiplegia on recovery from the anesthetic, due to the destructive action of the soap on the brain tissue, but otherwise remained well. The control animal developed poliomyelitis June 4, as proved by the microscopic findings.

Animal No. 15. Female. Javanese macaque. One attempt was made to immunize this animal with glycerinated virus on December 4, 1925, but was considered unsuccessful as the control animal did not develop the disease. The injection was performed subcutaneously in the back. April 17, 1926, the animal was injected along with No. 3 with 5.0 cc. of the same soap-virus mixture, but the injection was performed intraperitoneally rather than cerebrally. As indicated under the description for No. 3, the control developed the disease on the 5th day and was killed the 6th day.

Animal No. 17. Male. *Macacus rhesus.* May 25, 1926, was injected intraperitoneally with 4.0 cc. of fresh soap-virus mixture at the same time that animal No. 14 was treated. As described under animal No. 14 the control developed the disease June 4.

June 6, 1926, in order to test the possible immunity of the 4 animals, they were injected cerebrally with 1 cc. of fresh virus in physiologic saline along with 3 normal control monkeys; No. 10, male, Javanese macaque; No. 19, male, *Macacus rhesus,* and No. 21, female, *Macacus rhesus.* Control No. 10 was found dead July 12, 1926. Control No. 19 showed the first symptoms of poliomyelitis July 14 and was chloroformed July 16. Control No. 21 showed the first symptoms July 13 and was chloroformed July 16. The microscopic examination of the central nervous systems of the control animals showed the typical lesions of poliomyelitis in each case. The experimental animals No. 3, 14 and 15 all remained perfectly well. No. 3 died September 2 from an extensive pulmonary and visceral tuberculosis, as proved by autopsy. Microscopic examination of the brain and spinal cord revealed no evidence of poliomyelitis. Animals No. 14 and 15 are living and well at the present time. Animal No. 17 developed mild signs of poliomyelitis 13 days after injection, lived 15 days with the disease, and was chloroformed at this time to obtain autopsy material in which the acute lesions of poliomyelitis were found.

Since 3 of the 4 treated animals remained well, and only one developed a delayed and slow case of poliomyelitis, while the controls all died during an attack of poliomyelitis, the deduction is permissible that complete immunity was induced in 3 of the animals and partial immunity in the 4th. But the interpretation of this experiment must be conservative because certain facts vitiate its significance. Aside from the small number of animals experimented on, the most important of these is that we have been unable to produce the disease with more than 70 per cent to 75 per cent of the inoculations. On the other hand, in favor of the hypothesis that these animals were actually immunized, it is our general experience that if one animal of a number inoculated at a given time becomes infected, the rest of the controls usually succumb also, as in the final stage of the experiment just cited.

While this experiment is obviously inconclusive, it is just as obviously suggestive that sodium ricinoleate may be a medium for actively immunizing against poliomyelitis, and we intend to test this idea until we are satisfied that it is either true or false.

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[1] Larson, W. P., and Nelson, E., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 278. Larson, W. P., Evans, R. D., and Nelson, E., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 194. Larson, W. P., Halvorson, H. O., Evans, R. D., and Green, R. G., *Colloid Symposium Monograph*, 1925, iii, 152. Larson, W. P., and Eder, H., *J. Am. Med. Assn.*, 1926, lxxxvi, 998. Larson, W. P., Huenekens, E. J., and Colby, W., *J. Am. Med. Assn.*, 1926, lxxxvi, 1000. Larson, W. P., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 497.

3339

The Growth of the Spinal Axis of the Human Body in Prenatal Life.

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It has been previously pointed out[1, 2, 3] that the growth of the external dimensions of the human body in the fetal period is directly proportional to the growth in length of the body as a whole. The same law holds true for many of the lineal dimensions of the internal organs and parts, and is particularly well illustrated by the growth of the spinal column in prenatal life.

We have studied these relationships by measurements of the total length of the spine and its parts in 148 specimens ranging from 2.5 to 55.0 cm. in total or crown-heel length, and have placed the results in the form of empirical formulae. These formulae were computed from the mean spine lengths for 5 cm. intervals of crown-heel length by the method of averages, weighting by the square root of the number of cases in each interval.

In all instances we have found that the relationship of the spine and its parts with the total body-length is a rectilinear one which may be represented by the expression:

$$S = aL \pm b \quad (1)$$

where "S" is the length of the spine or any spinal segment, "L" is the total body-length and "a" and "b" are empirically determined constants.

The growth of the total spine is illustrated by the upper curve in figure 1 and may be expressed by the formula:

$$\text{T. S. (mm.)} = 0.4449 \text{ L (mm.)} + 4.46 \text{ mm.} \quad (2)$$

The calculated means of the spine length, as determined by this formula, show a mean weighted absolute deviation of 1.37 mm. from the corresponding observed averages, for the 5 cm. intervals of crown-heel length, which is approximately the same as the mean of the probable errors of these observed averages. The mean, weighted, relative deviation of the observed from the calculated values is 1.29 per cent.

The growth of the cervical spine (shown in the lower curve in figure 1) may be expressed by the formula:

$$\text{C. S. (mm.)} = 0.0830 \text{ L (mm.)} + 2.75 \text{ mm.} \quad (3)$$

The calculated means for the 5 cm. intervals of crown-heel length show a mean weighted absolute deviation of 0.40 mm., which again is approximately the same as the mean of the probable errors of the observed averages. The corresponding mean relative deviation is 2.52 per cent.

The growth of the thoracic spine is illustrated in the middle curve of figure 1 and may be expressed by the formula:

$$\text{Th.S. (mm.)} = 0.1722 \text{ L (mm.)} + 1.56 \text{ mm.} \quad (4)$$

The computed 5 cm. interval averages show a mean, weighted, departure of 0.88 mm. from the corresponding observed values, which is slightly more than the average of the probable errors of these observed means. The corresponding percentage deviations in this instance average 1.95.

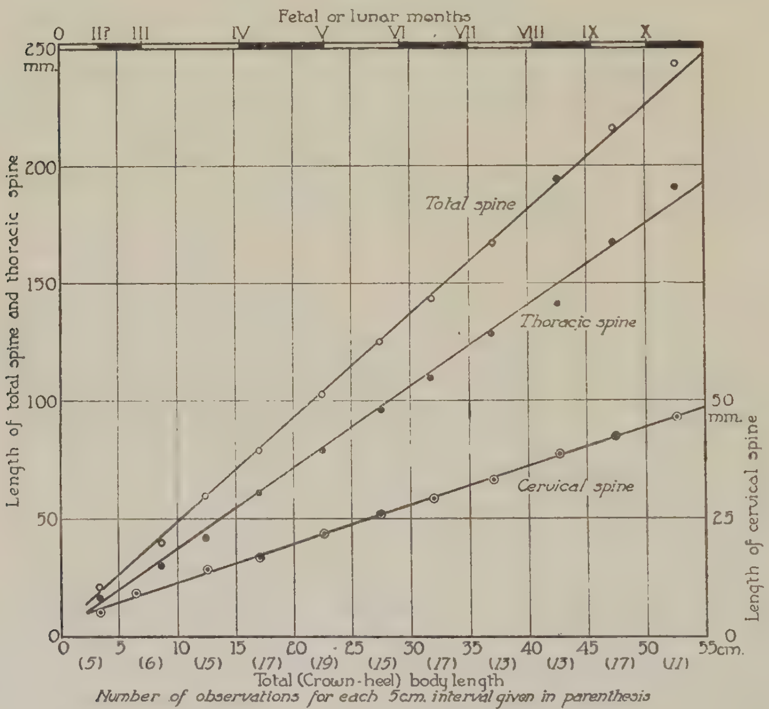
The growth of the lumbar spine may be represented by the empirical formula:

$$\text{L.S. (mm.)} = 0.1005 \text{ L (mm.)} - 0.27 \text{ mm.} \quad (5)$$

The mean, weighted, deviation in this instance is 0.22 mm., which is about half of the mean of the probable errors of the observed averages of this series. The mean weighted, relative deviation is 1.79 per cent.

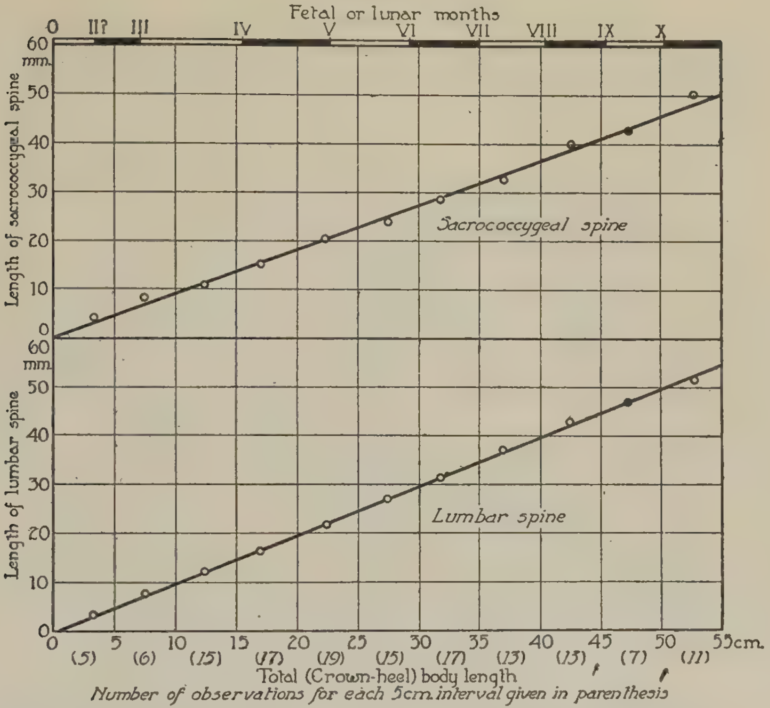
These figures indicate that the law of developmental direction holds good for the spine as a whole, and the various spinal segments, with the exception of the sacrum and coccyx, which show a relatively great growth in early prenatal life and a relatively slow velocity of growth thereafter. This exception falls in line with our knowledge of the phylogenetic reduction of the sacrocaudal vertebrate in the higher primates. A more detailed quantitative analysis of the rates of growth of the spinal column will be presented in the near future.

FIG. 1.



A graph illustrating the growth of the total spine, the cervical and the thoracic spine in prenatal life. The spine lengths (in mm.) are plotted against the total body-length (in cm.). The observed means for the 5 cm. intervals of body-length are represented by dots or circles, and the lines drawn through them represent the analytical expressions given in this paper. The total body-length is given on the base-line of the graph. The panels along the upper margin represent the age in fetal or lunar months. The latter are computed by the empirical formula of Scammon and Calkins[1] for the relation of fetal age to body-length (except for the value for two months which has been estimated by arithmetic interpolation from Mall's data[2]).

FIG. 2.



A graph illustrating the growth of the sacrococcygeal and lumbar spine in prenatal life. Symbols and conventions as in figure 1.

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[1] Calkins, L. A., *Anat. Record*, 1921, xxi, 47.

[2] Calkins, L. A., and Scammon, R. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 353.

[3] Akiba, T., *Folia Anat. Japonica*, 1924, ii, 89.

[4] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1924, xx, 353.

[5] Mall, F. P., *Man. Human Embryol.*, 1910, i, 180-201.

3340

The Growth of the Gastro-Intestinal Tract of the Human Fetus.

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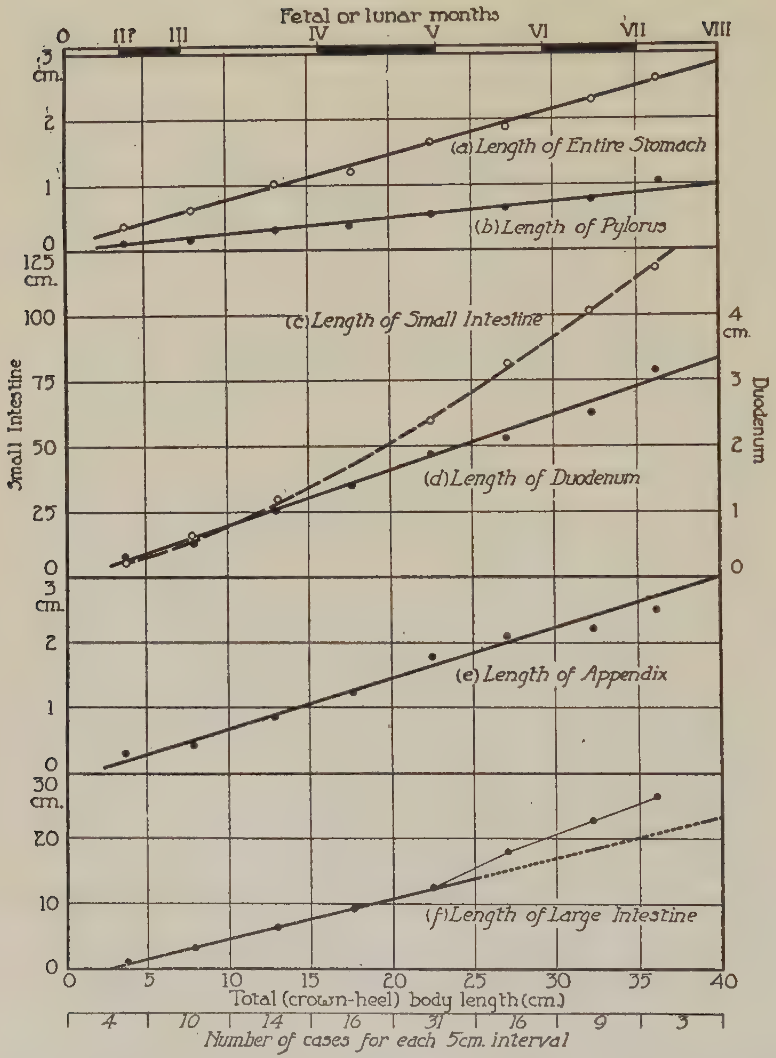
A quantitative study has been made of the lineal growth of the gastro-intestinal tract in human fetuses, ranging from 2.35 to 37.5

FIG. 1.



A graph illustrating the growth of the several segments of the gastro-intestinal tract in prenatal life. The lengths of the various parts of the gastro-intestinal tract (in cm.) are plotted against the total body-length (in cm.). The dots or circles represent the observed means for the 5 cm. intervals of body-length, and the lines drawn through them represent the analytical expressions given in this paper. The total body-length (in cm.) is given on the base line of the graph. The panels along the upper line represent the age in fetal or lunar months. The latter are computed by the empirical formula of Scammon and Calkins[1] for the relation of fetal age to body-length (except for the value for two months, which has been estimated by arithmetic interpolation from Mall's data[2]).

cm. in total or crown-heel length. One hundred and three preserved specimens were available for this study.

When the lengths of the stomach as a whole, of the pylorus, of the duodenum and of the appendix are plotted against the total body-length, the values follow the course of a straight line and may be expressed by the general formula:

$$D = aL \pm b \quad (1)$$

where "D" is the length of the segment of the digestive tract in question, "L" is the total body-length and "a" and "b" are empirically determined constants. The graphs of these relationships are shown in the following figure. The empirical formulae for these dimensions were determined from the 5 cm. interval averages of crown-heel length by the method of means, weighting by the square root of the number of cases.

The growth in length of the entire stomach, with respect to total body-length, may be represented by the empirical formula:

$$\text{S.L. (cm.)} = 0.0693\ \text{L (cm.)} + 0.079\ \text{cm.} \quad (2)$$

The mean weighted absolute deviation of the calculated values from the observed means is 0.049 cm., which is about one-fifteenth of the average interquartile measure of variability of the observed means used in this study.* The corresponding relative deviation is 4.17 per cent.

The growth in length of the pylorus may be represented by the empirical formula:

$$\text{P.L. (cm.)} = 0.0233\ \text{L (cm.)} + 0(0.0218) \quad (3)$$

The computed means by this formula show an average weighted departure of 0.015 cm. from the observed 5 cm. range averages. The mean weighted relative deviation is 4.0 per cent. The mean weighted deviation is approximately one-tenth of the average of the interquartile measures of variability of the observed means.

The growth in length of the duodenum may be represented by the empirical formula:

$$\text{D.L. (cm.)} = 0.0845\ \text{L (cm.)} - 0.0813\ \text{cm.} \quad (4)$$

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* As a rough measure of the variability of the observed means we have used the expression:

$$\frac{Q_3 - Q_1}{2\sqrt{n}}$$

where "Q_3" and "Q_1" are the third and first quartiles, respectively, of the distribution of cases forming the mean, and "n" is the number of cases. In a strictly normal distribution this value would be the probable error of the mean as usually computed. In distributions of the type formed by this material the measure is not equivalent to the probable error but is approximately the same magnitude although more variable.

The computed means show an average weighted deviation of 0.068 cm. from the corresponding observed ones. This is equal to approximately one-eighth of the mean of the interquartile measures of variability of the observed averages.

The growth in length of the appendix may be represented by the empirical formula:

$$\text{A.L. (cm.)} = 0.0796 \text{ L (cm.)} - 0.154 \text{ cm.} \quad (5)$$

The mean weighted deviation is 0.088 cm. and 9.12 per cent. The former is approximately one-seventh of the mean of the interquartile measures of variability of the observed averages.

The growth of the large intestine differs somewhat from that of the remainder of the digestive tract. Until the fetus reaches a total length of approximately 25 cm., the growth of the colon, with respect to body-length, is obviously rectilinear, but thereafter the large intestine length increases more rapidly. We think this change is due to the collection of meconium in the large intestine in the latter half of fetal life, and that the increased length is a mechanical rather than a true growth phenomenon. This is supported by the fact that the large intestine undergoes a noticeable reduction in length after the discharge of meconium following birth. The growth in length of the large intestine up to 25 cm. crown-heel length may be expressed by the empirical formula:

$$\text{L.I.L (cm.)} = 0.6136 \text{ L (cm.)} - 1.616 \text{ cm.} \quad (6)$$

The calculated means show an average weighted departure of 0.13 cm. This is approximately one-third of the mean of the interquartile measures of variability of the observed averages. The mean weighted relative departure is 5.26 per cent from the corresponding observed values.

The small intestine does not follow a lineal course of growth with respect to body-length, but may be represented quite accurately by a shallow curve having the formula:

$$\text{S.I.L. (cm.)} = 0.89274 \text{ L (cm.)}^{1.3598} \quad (7)$$

The values obtained by this expression show a mean weighted deviation of 2.5 cm. from the observed 5 cm. body-length interval averages which is approximately equal to the mean of the interquartile measures of variability of the observed averages.

These formulae show that the lineal growth of the gastro-intestinal tract, like that of most other parts of the body, follows the law of anteroposterior direction, for more cranial portions grow relatively more rapidly in the early part of prenatal life but diminish in velocity thereafter, while the successive caudal portions show smaller amounts of growth in the embryonic period but increasing relative velocities of growth thereafter.

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[1] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 353.

[2] Mall, F. P., *Man. Human Embryol.*, 1910, i, 180-201.

3341

Effect of Phosphorus Deficient Rations on Blood Composition in Cattle.

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The authors[1] have described elsewhere a condition in which numerous cattle in Minnesota suffer from a mineral deficiency which causes stunting, osteophogia, general pica, delayed oestrous and other serious metabolic disturbance. The animals are fed largely on prairie grass pasture or hay, depending on the season, with a limited amount of grain. An outstanding characteristic of the hay is its relatively low phosphorus content, which often falls below 0.2 per cent P_2O_5. / This fact points to insufficient phosphorus as the primary cause of the more or less disastrous results encountered. The further fact that bone meal or other forms of phosphate prevent and relieve the situation, when fed as a part of the ration, furnishes additional support for the phosphorus deficiency etiology of the disease.

We have recently obtained striking proof of these facts through a study of the inorganic Ca and P in the blood. The principal data secured to date are given in the accompanying table. All the animals had been reared in the P-deficient region and had been under experimental observation for at least 75 days when the first analyses were made. The hay fed during the period covered by the analyses as well as some months previous, was grown on mineral soil, low in phosphorus. The hay was of high quality for prairie hay; it had a bright green color and was palatable. The animals received all they would eat. The oats fed were of unknown origin, and were fed at the rate of 2 to 12 pounds daily, per head, depending on whether the cattle were dry or milking, and the milk production.

The P content of these prairie hay-oats diets was abnormally low.

The Ca content was not high but was apparently adequate. Typical cases were as follows, based on analyses of the products and monthly food intake: The ration of a dry cow contained 0.08 per cent P and 0.36 per cent Ca; that of a cow giving 12 pounds milk daily contained 0.13 per cent P and 0.30 per cent Ca; that of a dry cow receiving phosphorus supplement contained 0.28 per cent P and 0.35 per cent Ca.

TABLE I.

Animal No.	Mineral Supplement fed	Period of feeding mineral[1]	P and Ca in 100 cc. blood plasma during											
			August			September			October			November		
			P	Ca	Ca×P	P	Ca	Ca×P	P	Ca	Ca×P	P	Ca	Ca×P
		days	mg.	mg.		mg.	mg.		mg.	mg.		mg.	mg.	
33[2]	none		2.55	12.29	31.2	2.20	13.46	29.6	2.99	12.51	37.4	1.75	12.81	22.4
73	none		2.47	12.13	30.0	2.11	13.49	28.5	2.28	12.87	29.2	1.99	12.25	24.4
91[2]	none		2.39	11.87	28.3	1.93	12.82	24.7	1.26	11.78	14.5	1.03	11.60	11.9
94[2]	none		1.28	13.01	16.6	2.04	15.2	31.1	1.32	16.02	21.1	1.09	10.97	11.9
75	$CaCO_3$[3]	102	1.75	12.19	21.3	1.80	14.63	26.3	1.94	13.30	25.8	2.38	12.62	30.0
93[2]	$CaCO_3$	73	1.07	12.06	12.9	2.02	14.6	29.6	1.03	16.47	16.9	1.58	13.17	20.8
58	NaH_2PO_4[4]	600	6.56	11.29	74.1	5.43	13.11	71.2	—	—	—	—	—	—
74	NaH_2PO_4	102	5.51	10.65	58.7	5.17	11.78	60.9	5.40	11.63	62.8	4.75	10.53	50.0
92[2]	NaH_2PO_4	85	4.41	12.66	55.8	3.82	12.86	49.1	5.27	12.06	63.6	6.04	10.51	63.5

[1] Up to time of 1st analysis.

[2] Cow in milk.

[3] 100 grams daily for cows 75 and 93.

[4] **75 to 100 grams daily for cows 58, 74, and 92.**

Blood Ca was determined* by the Kramer and Tisdall[2] method, P by the Briggs[3] modification of the Bell-Doisy method, in both cases on citrated plasma. It was found necessary to add a known quantity of phosphate to the low P samples before a satisfactory estimation could be made. The analyses reported represent the changes in succeeding months based on the average of samples of venous blood taken on three successive days.

The data show clearly the abnormally low inorganic P in the blood plasma of animals confined to the hay-oats ration, either with or without $CaCO_3$ supplement. The plasma Ca, however, was normal throughout. / The product of Ca × P for these animals is seen to be rarely above 30 and frequently much below 20, values which would be indicative of a rachitic condition in growing animals according to the results of Howland and Kramer.[4] In contrast is the normal composition of the blood of animals fed the hay-oats ration plus NaH_2PO_4. The Ca × P in these cases ranges from 50 to 75.

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[1] Palmer, L. S., and Eckles, C. H., *Minn. Agr. Exp. Station Bulletin* 229, 1926.

* The analyses were made by W. M. Neal, analyst.

[2] Kramer and Tisdall, *J. Biol. Chem.*, 1921, xlvii, 475.

[3] Briggs, *J. Biol. Chem.*, 1924, lix, 255.

[4] Howland and Kramer, *Am. J. Dis. Child.*, 1921, xxii, 105.

3342

Filtration Through Living Membrane.

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The mesentery of the bullfrog *(Rana catesbiana)* was used as the filtration membrane to test the rate of filtration of various fluids. The mesentery was so placed that a funnel could be easily approximated to it. This funnel led into a flask from which a glass tube connected with the vacuum pump and manometer and regulating stop cock. The animal was anesthetized with urethane. A catheter could be introduced into the bladder or into each ureter. This technic can also be applied to similar membranes in other animals.

During the experiment the flow of blood could be observed. Pulsation in some cases was readily visible at a pressure of 40 cm. of water. Even when no pulsation was visible, in some vessels at least, the flow of blood could be seen. Some membranes readily stand a pressure considerably in excess of 700 mm. of mercury; none broke at a pressure of less than 180 mm. of mercury.

The lack of uniformity in the rate of filtration with membranes of different frogs, and also the lack of uniformity in the slowing of the filtration, has necessitated thus far relatively short experiments and the counting of drops. The initial rate of filtration can frequently be restored by releasing the pressure at intervals and allowing the membrane to rest a few minutes. As a rule, with increased pressure there was an increase in the rate of filtration, but the relationship was not mathematical.

If the 0.8 per cent solution of sodium chloride is cold, the rate of filtration may decrease one-half, but will return to the initial rate if the fluid is warmed again. The difference in rate of filtration of fluids at room temperature and at higher temperatures was not striking.

Acid or alkali was added to the solution of sodium chlorid within a range of pH 2.2 to pH 9 and more. On the whole, filtration of the more acid solutions was slower. A 10 per cent solution of sodium chloride filtered more slowly than a 0.8 per cent solution; a saturated solution of sodium chloride caused extravasation of blood, and filtration was slowed, but could be increased by washing with a 0.8 per cent solution. A half saturated solution of ammonium sulphate or a 50 per cent solution of glucose changed the membrane visibly.

The mesentery was permeable to hemoglobin and Congo red, and other dyes. It held back milk globules, letting through the caseinogen. There seemed to be differences in the rate of filtration of various protein solutions. When uritone was placed in the lumen of the intestinal loop surrounding the mesentery, the filtrate gave a positive formaldehyd reaction. Phenolsulphonephthalein thus injected did not appear in the filtrate, but did appear when injected intravenously, as did mercurochrome. Intravenous injection of 50 per cent glucose solution was followed by its appearance in the filtrate, but the rate of filtration of the sodium chloride was considerably reduced.

The process is not one of filtration alone. Protein always appeared in the filtrate, but apparently to a lesser degree when water was used as the filter fluid. Some calcium was also found in the filtrate. The membrane was alkaline to litmus. Filtrate of solutions of sodium chloride was alkaline, and phenolsulphonephthalein came through alkaline.

The method may lend itself to the study of absorption at the same time.

Missouri Branch.

St. Louis University School of Medicine, December 15, 1926.

3343

The Action of X-Rays on the Organism.

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X-rays can produce cancer as well as destroy it. The action of X-rays, therefore, will be understood only when we have first understood the mechanism of growth in tissue cells. One of us had shown[1] that the protoplasm is a colloidal fluid substance which reacts differently according to its immediate state of aggregation. The state of its aggregation is regulated by a substance or substances which is formed by the cells. This substance or substances has been called the archusia (S). In low concentrations (S^1) the archusia has no effect. In medium concentration (S^2) it causes the cells to migrate, to coagulate extracellular proteins, engorge themselves with proteins and fat particles, or to function. In high concentrations (S^3) the cell digests these proteins and fats, absorbs water, grows and divides by mitoses. In all higher concentrations (S^4) it causes the cells to disintegrate or suffer self-digestion.

In the growth of the cell protein synthesis is not the only essential reaction, but the cell must also acquire or form a lipoid substance, the ergusia. This substance is essential for maintaining the structure of protoplasm. It is an active coagulant of protein. It is liberated by the cells in an (S^2) concentration of the archusia. It is the active agent in the specific absorption of water and other substances necessary for growth, and the surface tension lowering substance necessary for the migration of the cells. The ergusia in excess in the tissue inhibits growth, as any substance, formed in any incomplete reaction, inhibits the reaction. It is also liberated by cells when they disintegrate as the result of a high concentration (S^4) of the archusia.[2]

The cells do not form the ergusia in excess. It exists in excess in the normal organism. The excess is supplied in the form of vitamin A.[3] The cells also do not form and retain ample archusia for growth, except when they are crowded, as in cancer. The archusia in the normal organism is also supplied in part from without as vitamin B.[4]

Having ascertained these facts, it became of interest to study the effect of X-rays on animals fed on normal diets, diets deficient in either vitamins A or B, and diets balanced in these vitamins, but deficient in other vitamins necessary for a normal existence.

Carefully graded doses of X-rays (Ernst's method)[5] were used in treating these animals. The results of these experiments have shown that the life of rats, fed on a diet deficient in vitamin B, is increased by giving 10 milliampere minutes (13 "e" units) of X-rays twice a week. This dose has no effect on rats fed on a diet deficient in vitamin A.[6] Twenty-five and 50 milliampere minutes (34 "e" units and 68 "e" units respectively) increase not only the length of life, but also the growth of rats fed on both a minus A and a minus B diet,[7] while larger doses, 75 and 100 milliampere minutes (102 "e" and 136 "e" units respectively) cause rapid deterioration and death of these animals.[8]

It has been known for a very long time that many toxic substances show evidence of stimulation in small doses. X-rays evidently belong in this class. Their action as noted from these observations, is one of molecular disintegration. In small doses X-rays stimulate in regular sequences those molecular disintegrations which are the source of the normal energy for life. In larger doses they cause the disintegration of the more important molecular constituents of the cell. It is also interesting to note that moderate doses of X-rays act very much the same as ultraviolet light, in that they liberate vitamin A from tissues absorbing it so that this vitamin can be used by other tissues in the organism.[6]

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[1] Burrows, M. T., *Am. J. Anat.*, 1926, xxxvi, 289.

[2] Burrows, M. T., and Jorstad, L. H., *Am. J. Physiol.*, 1926, lxxvii, 38.

[3] Burrows, M. T., and Jorstad, L. H., *Am. J. Physiol.*, 1926, lxxvii, 24.

[4] Ernst, E. C., *Radiology*, 1925, v, 468.

[5] Burrows, M. T., Jorstad, L. H., and Ernst, E. C., *Radiology*, 1926, vii, 279.

[6] Burrows, M. T., Jorstad, L. H., and Ernst, E. C., *J. Am. Med. Assn.*, 1926, lxxxvii, 86.

[7] Burrows, M. T., Jorstad, L. H., and Ernst, E. C., to appear in *Radiology*.

3344

The Differential Action of Pressure on Fibers of Different Sizes in a Mixed Nerve.

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The effect of pressure on a mixed nerve was studied to determine whether it would shed any light upon the nature of the complex constitution of nerve trunks. Nerve action potentials recorded at a distance from a stimulating electrode have been shown to have a form dependent on the structure of the nerve as revealed histologically. The velocity of conduction in the fibers varies as the diameter; therefore in the action potential wave, recorded as above, the first of the action wave is made up by potentials in large fibers, and the end by potentials in small fibers.

A priori mechanical considerations lead one to suppose that an applied external pressure would block the large fibers before the small ones. This was indeed found to be the case. The nerve was blocked by a method similar to that of Meek and Leaper[1] who first applied fluid pressure through a cuff. A brass T-tube was made 12 mm. long with a bore of 2.5 mm. Through this was threaded a rubber tube with a fairly thin wall, whose outside diameter fitted the bore of the tube. The ends of the rubber tube were everted over the ends of the brass tube and tied, then covered with a brass cap to prevent longitudinal extension of the cuff. The nerve was threaded through a perforation in the cap. Pressure was then conveniently applied from an oxygen cylinder, being accurately controlled by a reducing valve. It was applied between the stimulus and the leads to the recording instrument, a Braun tube oscillograph; therefore, what is obtained is a record of normal waves, the form of the wave being altered either through block in some of the fibers, or a delay in traversing the compressing chamber.

When pressure is rapidly applied, the front of the action potential wave as it appears on the fluorescent screen, seems to melt away. When pressure is applied more slowly a long series of gradations is demonstrable. The α wave gets smaller, then disappears. But before this is completed the β wave shows a delay in passing the cuff, then the fibers in it too begin to be blocked, and later the same cycle is repeated with respect to the γ wave.

The significance of these experiments is revealed by comparing them with some old subjective observations on block. To quote one case; Goldschneider[2] found that the order in which skin sensations disappear, when the innervation is blocked by pressure, is cold, contact, heat and pain. Therefore a comparison of the changes in the action potential wave with such a series of observations gives an indication of the relative sizes of the fibers carrying the different modalities; and it, together with similar comparisons using data obtained by other methods of blocking nerves, should aid, when compared with the corresponding losses of function, in the analysis of a nerve trunk for the functions of its various fibers.

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[1] Meek, W. J., and Leaper, W. E., *Am. J. Physiol.*, 1911, xxvii, 308.

[2] Goldschneider, *Pfluger's Arch.*, 1886, xil, 115.

3345

Skin Reactions to Scarlatinal Streptococcus Filtrate in New-Born Infants and Their Mothers.

JEAN COOKE.

From the Department of Pediatrics, Washington University School of Medicine, and the St. Louis Children's Hospital.

Observations on over 150 infants during the first 2 weeks of life, and their mothers, were made by noting (1) the reaction of the skin to 2, 20 and 50 standard skin test doses of scarlatinal toxin, (2) the occurrence of antitoxin in the blood of a number of these infants and their mothers, and (3) later skin tests on a number of the same infants between the ages of 6 weeks and 2 months.

It was found that the skin of infants in the first 2 weeks of life does not react to small amounts (2 S. T. D.) of toxin, only one instance of a positive reaction having been observed. With larger amounts (20 and 50 S. T. D.) only a small proportion (about one-eighth) show positive reactions, and the mothers of these babies in practically every instance have positive reactions to two skin test doses. It was quite exceptional for an infant to give a positive reaction to 20 or 50 S. T. D. if the mother was negative to 2 S. T. D. The presence of antitoxin in infant's serum had no relation to the infant's skin test, but is associated with the presence of antitoxin in the mother's blood. No antitoxin was demonstrated in infants of mothers who reacted to 2 S. T. D., but it was rather constantly present in infants whose mothers gave negative reactions to 2 S. T. D., even though skin tests with larger amounts were positive.

On later examination of the infants at the age of 6 weeks to 2 months, the skin reaction had become positive in certain babies, to the larger amounts (20 to 50 S. T. D.) of toxin and occasionally to 2 S. T. D. This tendency was most marked in those whose mothers reacted to 2 S. T. D., less marked in those who required 20 S. T. D. to give a reaction, and still less in those who required 50 S. T. D. None of the infants whose mothers were negative to 50 S. T. D. became positive with 50 S. T. D. or less during the first two months of life.

Apparently, the skin of new born infants does not react to small amounts of scarlatinal toxin, and reacts to larger amounts in only a small proportion of cases. If, later, the baby's skin develops a positive reaction, the test is positive first with larger amounts of toxin and later with small amounts. The skin reactivity develops earlier in infants with no antitoxin in the blood, at birth, than in those whose blood contains antitoxin.

3346

Relation of Skin Reactions to Scarlatinal Streptococcus Filtrate in Children, to Antitoxin in Blood.

JEAN COOKE.

From the Department of Pediatrics, Washington University School of Medicine, and the St. Louis Children's Hospital.

A large number of children over 3 years of age have been given skin tests with varying amounts of a standard scarlatina streptococcus filtrate toxin. In a group giving a positive Dick test with 1 or 2 S. T. D., no antitoxin was demonstrable in the blood. In a few children who were negative to 2 S. T. D., but positive to 5 S. T. D., the antitoxin determinations were somewhat irregular, although when 10 or 20 S. T. D. of toxin or more was necessary to obtain a skin reaction, antitoxin was readily demonstrable, and in those children who gave negative skin tests to 100 or 400 S. T. D., antitoxin was present in the blood in rather large amount.

In tests on younger children a similar association of antitoxin with negative skin tests was found. A certain number of infants, more commonly in the first year, but occasionally later, who gave negative skin reactions to 20 or 50 S. T. D. of toxin, had no antitoxin demonstrable in the blood.

The association of the presence of antitoxin in the blood with negative skin tests to small amounts of toxin, which seems fairly constant in older children, is somewhat more variable in infancy, and certain older infants may have negative skin tests, even to considerable amounts of toxin, without demonstrable antitoxin in the blood.

3347

Action of Formaldehyde Upon Physiologically Active, Histamine-like Substance Produced by Gas Bacillus.

ARTHUR ISAAC KENDALL.

From the Department of Bacteriology and Public Health, Washington University School of Medicine, St. Louis, Mo.

The phenomenon of contracture, induced in surviving sections of guinea pig intestine, suspended in Tyrode solution, by the addition of very small amounts of gas bacillus culture[1] suggests that the active substance, possibly substances, contains one or more free ethylamine groups, attached to an aromatic nucleus of the general type R :aromatic nucleus :ethylamine.

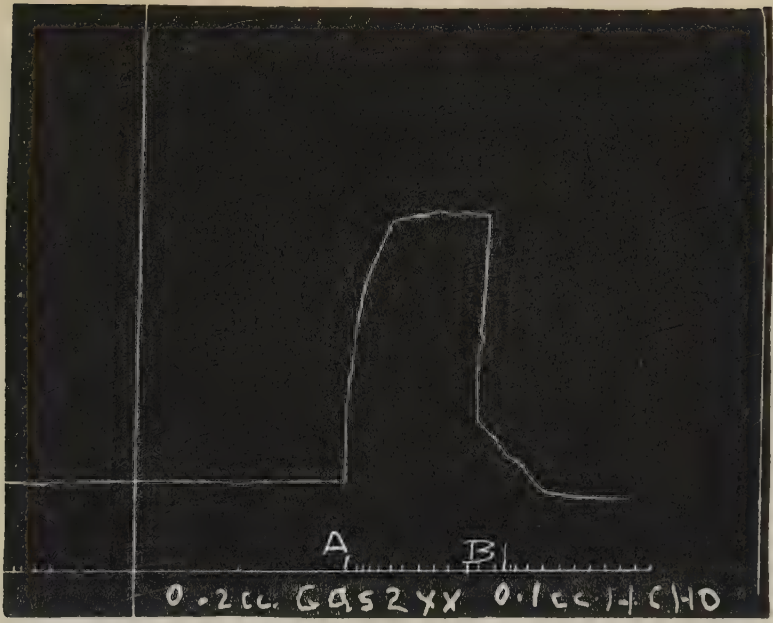
In such a compound, current theory predicates that its physiological activity is intimately associated with the presence of the free aromatic ethylamine group. It follows theoretically that any chemical change involving the elimination of this free NH_2 group from such a compound should reduce, or even destroy the physiological action of the molecule as a whole.

The addition of formaldehyde to primary amines changes them according to the following equation, in accordance with the well known "formol titration" of Henriques and Sörenson :[2]

$$R.CH_2.CH_2.NH_2 + H.CHO = R.CH_2.CH_2.N{:}CH_2 + H_2O.$$

It has been found by actual experiment that the addition of 0.1 cc. of neutralized formalin solution releases the contracture in a piece of guinea pig intestine which has been induced by either the soluble, physiologically active substance found in cultures of the gas bacillus, or by histamine. The following graph is the record of such a contracture induced by 0.2 cc. of gas bacillus filtrate, and released by 0.1 cc. of formaldehyde solution. Precisely similar ones have been obtained with histamine and formaldehyde.

The formaldehyde-ethylamine compound may be washed out of



the preparation with fresh Tyrode solution, and contracture again induced, indicating that the intestinal muscle is not materially injured by the several reagents.

The experiments seem to indicate that contracture of smooth muscle may be induced by an aromatic ethylamine group, and that chemical change of this ethylamine group may thereby remove the contracture-inducing capacity of the entire molecule.

If 0.2 cc. of formaldehyde solution is added to the Tyrode solution in which the intestinal strip is suspended, and then either gas bacillus filtrate, or histamine be added, no contracture is induced until enough of the latter is present to bind the formaldehyde, with an excess, which then induces contracture. In this regard, the reaction between the ethylamine group and the formaldehyde seems to be fairly quantitative.

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[1] Kendall, A. I., and Schmitt, F. O., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 104.

[2] Henriques, V., and Sörensen, S. P. L., *Zeit. f. Physiol. Chem.*, 1909, lx, 1.

3348

Tonus Measurements on Quadriceps Femoris Muscles in Man Before and After Lumbar Sympathectomy.

ALBERT KUNTZ.

From the St. Louis University School of Medicine, St. Louis, Mo.

Through the courtesy of Drs. G. E. Brown and A. W. Adson, and the Mayo clinic, the writer was privileged to study the tonus of the muscles of the lower extremities of 4 patients who underwent bilateral extirpation of the lumbar sympathetic trunks, including the second, third, and fourth lumbar ganglia. This operation effectively eliminates the sympathetic innervation of the muscles of the lower limbs.

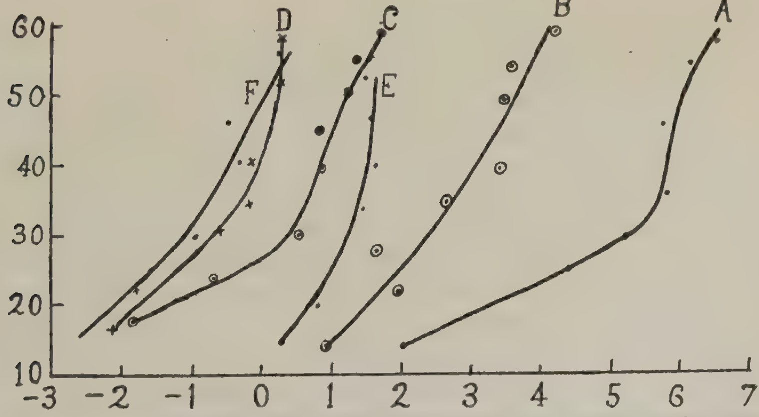
Tonus measurements, *i. e.,* measurements of the resistance offered by the muscle to passive extension, were carried out, according to Spiegel's method,[1] on the quadriceps femoris muscles. The results of these measurements are expressed in tonus curves. Two of the patients were available for study both before and after operation. In these cases tonus curves of both quadriceps muscles were obtained both before and after sympathectomy. The other patients were available for study only after operation. One of the latter was subjected to the tonus measurements 6 days after operation. The other had undergone the operation 1 year previously.

The patient studied a year after sympathectomy is a young woman who underwent the operation for the relief of Raynaud's disease. The other three patients are men afflicted with *thromboangiitis obliterans* in both lower extremities. The studies carried out on these patients revealed no appreciable changes in muscle tonus referable to the disease process. The tonus curves obtained before sympathectomy lie well within the range of normal variation.

Tonus curves of normal extensor muscles, under normal innervation, obtained by the method employed, rise slowly at the beginning and then more rapidly as the length of the muscle is increased by passive extension. The slow rise of the curve at the beginning (Fig. 1, A and C) illustrates the "brake phenomenon" which is characteristic of extensor muscles, while in a postural attitude in which they are near their minimum normal length. In the present study the measurements were initiated when the angle of the leg with the horizontal was less than 20°. The thigh remained in the horizontal position throughout the procedure. The curves obtained before sympathectomy, in the cases of both patients studied before operation, rise slowly until the angle of the leg with the horizontal approaches 30°, after which they rise more rapidly. These curves, two of which are illustrated in Fig. 1, A and C, are essentially normal tonus curves.

The tonus curves obtained after sympathectomy, in all the patients studied, rise rapidly from the beginning. Curves A and B, Fig. 1, are the tonus curves of the right quadriceps of one of the male patients, obtained before and after operation, respectively. Curves C and D are the corresponding curves respectively of the right quadriceps of another male patient. These curves indicate not only that the tonus of the muscle is materially diminished, following elimination of its sympathetic innervation, but also that it no longer exhibits the brake phenomenon, except possibly in a very slight degree. Curve E is the tonus curve of the left quadriceps of the third male patient, based on measurements carried out 6 days after operation. Curve F is the tonus curve of the left quadriceps of the female patient, based on measurements carried out 1 year after operation. These curves, like the tonus curves obtained immediately after sympathectomy, rise rapidly from the beginning, indicating that the muscles no longer exhibit the brake phenomenon,

FIG. 1.



A. Tonus curve of right quadriceps femoris obtained just before lumbar sympathectomy.

B. Tonus curve of the same muscle obtained just after lumbar sympathectomy.

C. Tonus curve of right quadriceps femoris obtained just before lumbar sympathectomy (another patient).

D. Tonus curve of the same muscle obtained just after lumbar sympathectomy.

E. Tonus curve of left quadriceps femoris (another patient) obtained six days after lumbar sympathectomy.

F. Tonus curve of left quadriceps femoris (another patient) obtained one year after lumbar sympathectomy.

except possibly in a very slight degreee. They also indicate diminution of muscle tonus; however, in the absence of tonus curves based on measurements carried out before the operation, the exact diminution of tonus referable to sympathectomy, in these cases, cannot be ascertained.

The possible rôle of increased circulation through the limb, following sympathectomy, in the diminution of muscle tonus, will be considered in another paper. The data available at present do not warrant the conclusion that circulatory changes referable to sympathectomy play more than a minor rôle in the measurable changes in muscle tonus following this operation.

The results of the tonus measurements on the quadriceps femoris in man here reported in general corroborate the results of our studies[2] involving tonus measurements on extensor muscles of the extremities, before and after sympathectomy, in experimental animals.

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[1] Spiegel, E. A., *Z. ges. neurol. u. Psych.*, 1923, lxxxi, 246.

[2] Kuntz, A., and Kerper, A. H., *Am. J. Physiol.*, 1925, lxxvi, 121. PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 77; 1926, xxiii, 367; 1926, xxiv, 103.

3349

Studies on the Enzyme-Action of Yeast.

MICHAEL SOMOGYI. (Introduced by G. H. Bishop.)

From the Laboratory of the Jewish Hospital of St. Louis, St. Louis, Mo.

If a suspension of yeast is brought together with a solution of glucose in adequately chosen proportions, then some protein admixed, and precipitated by a suitable protein precipitant, the glucose is carried down with the protein and yeast, and no trace of glucose can be recovered in the filtrate.

If, for example, 50 cc. of a 0.2 to 0.4 per cent solution of glucose and 10 g. of yeast, suspended in 50 cc. of diluted egg white or neutral casein solution, are poured together and mixed, subsequent precipitation by tungstic acid will remove all the glucose from solution. And this reaction takes place, even though fermentation be prevented by chilling both the yeast and the glucose to 0° C. before they are united, and kept at that temperature throughout the entire operation. Evidently *an adsorption-like combination takes place between yeast and glucose as soon as they come into contact.*

If the yeast is separated from a yeast-glucose solution by centrifugation or filtration, the glucose is recovered in the solution. On dialysis the glucose passes through the membrane. These observations indicate that the union between yeast and glucose is so labile that it cannot withstand the disrupting forces involved in these operations, and even might cast doubt on the conception of a union between yeast and glucose.

But on our further inquiry into the nature of the initial yeast-glucose combination, it was found that factors which damage enzyme action invariably also destroy or prevent the formation of this combination. Thus, in the presence of phenol, sodium fluoride, salycylic acid, etc., which prevent fermentation, the glucose is not removed from solution with the yeast-protein precipitate. Heating of the yeast-glucose mixture, too, prevents the precipitation of glucose. The result is the same if mercury solutions are used for the precipitation of the proteins in the mixture; namely, the glucose is recovered in the filtrate, whereas precipitation by tungstic acid—all other conditions being identical—removes the glucose from solution.

Further experiments have shown that the adsorption by yeast is specific and selective, in that it affects exclusively substances which are fermentable. Lactose, arabinose, etc., for instance, are not adsorbed, and can be quantitatively recovered under circumstances which lead to complete removal of glucose, sucrose, mannose. This specific selectivity of the adsorption phenomenon, plus the fact that substances which inactivate enzymes prevent it, indicate that it is a reaction between enzyme and substrate, in fact, the first phase of enzyme action.

The extreme rapidity of the reaction allows the inference that some of the enzymes of the yeast are situated in some definitely organized system around the surface of the cells; this may furnish the explanation for the great difference between the fermentation process by living yeast and cell-free fermentation, and further studies will be made on this question.

Using for protein precipitation in yeast-glucose mixtures tungstic acid and mercury, under otherwise identical conditions, enables us to follow up simultaneously both processes: the loose combination as well as changes which result in transforming sugar into non-reducing products. Results of these studies will be presented in a subsequent paper.

The simplicity and rapidity of the selective removal of glucose from solutions, as outlined in the foregoing, has served in our hands as the basis of a method for the quantitative determination of the "true sugar" in blood, urine, etc., and for the separation of fermentable sugars from non-fermentable ones. The method will be described elsewhere.

Pacific Coast Branch.

Lane Hospital, San Francisco, December 15, 1926.

3350

Iodine and Pulse Rate of Normal Individuals.

J. MARION READ, P. J. WALKER AND A. C. McKENNEY.

From the Medical Division of the Stanford University Medical School.

The belief exists and the statement has been made that the administration of iodine to normal individuals will produce slowing of the pulse rate. Since no satisfactory information upon this point could be obtained from text-books upon pharmacology and *materia medica,* it was determined to ascertain the effect on the normal pulse of ingesting the same amount of iodine as we have been administering to patients with Graves' disease.

Twelve internes, laboratory workers and physicians, without signs or symptoms of thyroid disease, and with normal basal metabolic rates, volunteered as subjects. There were 4 females and 8 males, 22 to 36 years of age. The pulse rate before arising in the morning was recorded for 10 days to ascertain the normal for each individual. On the next succeeding 10 days Lugol's solution (10 drops) was taken once daily before breakfast. During this period and continuing through another period of 10 days after taking iodine the pulse rate was recorded as in the preliminary period. Records of the mid-day and evening pulse rate were also obtained but these

TABLE 1.

	No.	Before			During			After		
		Mean	Range		Mean	Range		Mean	Range	
Women	4	60.77	44-70		62.18	48-74		63.3	52-78	
Men	8	64.91	50-76		64.8	50-90		65.3	50-80	
			Prob. Error	Stand. Devia.		Prob. Error	Stand. Devia.		Prob. Error	Stand. Devia.
Both Sexes	12	63.4	.425	6.561	64.2	.499	7.8	64.6	.44	6.76

proved to be so variable, depending upon the subjects' activity, that only the mean readings of the morning (basal) pulse are shown in Table I.

There was very slight increase in the pulse rate in 7, no change in 3 and a slight decrease in 2 cases. In no individual case was any marked effect noted, neither do the means for each sex show significant change in value. The general tendency of the means for all subjects is toward an increase, but the changes are inconsequential, when tested by the criterion that the difference in two values to bear significance must be greater than 3 times the probable error.[1] From the results of this small series it is concluded that iodine in the form of Lugol's solution, 10 drops once daily, has no appreciable effect on the pulse rate of normal individuals.

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[1] Pearl, Raymond, introduction to *Medical Biometry and Statistics*, 1923, Philadelphia (p. 214).

3351

One Solution Technique for Direct Microscopic Counting of Bacteria in Milk.

R. W. NEWMAN. (Introduced by C. S. Mudge.)

From the Dairy Laboratory, Division of Chemistry, State Department of Agriculture, Sacramento, Calif.

A method has been devised for simplifying the direct microscopic method of Breed for counting bacteria in milk.

In the official Breed method, 0.01 ml. of milk is spread over an area of 1 square centimeter on a glass slide. When dry, the slide is immersed in xylene for at least one minute (to remove the fat from the milk); then dried, immersed in 90 per cent alcohol for one or more minutes (to fix the smear to the slide); dipped in Loeffler's methylene blue until the milk smear is overstained; washed in water; decolorized in alcohol; again washed in water; dried and examined under a standardized microscope.

The new method eliminates the use of two solutions, two Coplin jars and four separate operations, and substitutes a single solution which simultaneously dissolves out the fat; fixes the milk smear to the slide and stains the bacteria and leucocytes. Excellent contrast between bacteria and background is secured without recourse to decolorization. Any one of the following solutions may be used:

Formula I.

Methylene blue, pwd.,	2.0	gm.
Ethyl alcohol, 95 per cent	60.0	ml.
Xylene, cp.,	40.0	ml.
Acetic acid, glacial	6.0	ml.

Dissolve methylene blue* in warm (70° C.) alcohol, adding the powdered stain very slowly. Then add the xylene and acetic acid. Filter. Keep in tightly stoppered bottle.

1. Milk smears are made on a glass slide in the usual manner. When dry, the slide is immersed in the above solution for ½ to 4 minutes.

2. Remove the slide from the solution and allow it to drain until the *smear* is thoroughly *dry*. In warm weather, this seldom requires longer than half a minute.

3. Wash *thoroughly* in water.

Formula II.

Methylene blue*, pwd.,	1	gm.
Ethyl alcohol, 95 per cent	54	ml.
Tetrachlorethane, tech.†	40	ml.
Acetic acid, glacial	6	ml.

Add the alcohol to the tetrachlorethane in a flask and bring to a temperature not to exceed 70° C.; add to the powdered methylene blue. Shake vigorously until the dye is completely dissolved; then add the glacial acetic acid. Filter the entire volume through a 15 cm. filter paper. Keep in tightly stoppered bottle.

Directions or Use.

1—Prepare milk smear. When dry
2—Immerse the smear in the solution and withdraw immediately.
3—Drain until dry (about 30 seconds).
4—Wash in water.
5—Dry and observe.

Formula III.

Methylene blue,* pwd.	0.7	gm.
Ethyl alcohol, 95 per cent	30.0	ml.
Tetrachlorethane, tech.†	40.0	ml.
Pyridine	15.0	ml.
Amyl acetate	15.0	ml.

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* Certified.

† Can be obtained from Eastman Kodak Co. Technical tetrachlorethane is relatively inexpensive.

Add the warm mixture of alcohol and tetrachlorethane to the powdered methylene blue and shake vigorously until dissolved. When cool, add the pyridine and amyl acetate and filter. Keep in tightly stoppered bottle.

The same staining directions are followed in using this last solution as are given under Formula II.

Formula III, which is an alkaline solution, is submitted to meet any possible objection to the use of an acid-methylene blue stain. Pyridine is incorporated as an accessory fixative agent. The function of the amyl acetate is to partially mask the odor of pyridine.

A series of comparative counts made in the laboratory on raw and pasteurized milk of high and low count revealed no apparent variation from the official Breed method.

Although the use of Formula I has been found very satisfactory, it is believed that because of the added advantages possessed by Formulae II and III, they will be found more satisfactory and for this reason are recommended.

3352

Presence of Physiologically Active Substance in Two California Species of Ephedra.

GUY W. CLARK AND G. W. GROFF.

From the Division of Biochemistry and Pharmacology, Medical School, University of California, Berkeley, and the Agricultural College, Lingnan University, Canton, China.

Current interest in Ma Huang,[1] the Chinese drug plant *Ephedra*, makes imperative a study of the various species of the genus, especially with respect to the activity and nature of the chemical principles which they yield.

The problem is complicated by the lack of knowledge concerning the effect of species variation and ecological conditions upon the yield of physiologically active substances.

A complete study involves: (1) Determination of the species and varieties which contain physiologically active substances. (2) The isolation and identification of the active principles. (3) The experimental and clinical testing of the pure principles obtained.

In the work reported in this paper our efforts have been confined to a comparative study of the activity of *Ephedra californica*

Wats and *Ephedra nevadensis Wats,* using for comparison *Ephedra* obtained from Chinese drug shops in San Francisco. We have also used an ephedrine sulphate solution prepared by Eli Lilly Company, and a sample of the pure hydrochloride obtained from the Peking Union Medical College.*

Ten per cent infusions of the crude drug have been prepared in a number of ways: water, 0.9 per cent NaCl, 50 per cent ethyl alcohol, 50 per cent ethyl alcohol containing 1 per cent tartaric acid, and water slightly acidified with H_2SO_4. In all cases the solutions were heated on a steam bath 3 to 4 hours. Prior to use, the alcohol extracts were evaporated and original volume restored with 0.9 per cent NaCl. These solutions, kept in tightly stoppered pyrex flasks, retained their original activity up to 19 days, when the experimental work reported here was concluded.

Blood pressure experiments with a number of rabbits indicate that certain specimens of *Ephedra californica Wats* contain a small amount of pressor material. The pressor effects obtained with the extracts of *Ephedra nevadensis Wats* were much more pronounced than those obtained from either the *Ephedra californica* or the material from the Chinese drug shop. In one experiment four separate intravenous injections (1 cc. of the *Ephedra nevadensis* infusion per kilo) gave the following changes in blood pressure† (1) 64 mm., (2) 54 mm., (3) 52 mm., (4) 44 mm. A similar injection of the infusion of the Chinese drug, given between (2) and (3) caused a rise of only 14 mm. Thirty mgm. of the pure hydrochloride (2), given intravenously after (4), caused a rise in blood pressure of 34 mm., which persisted for much longer time. The infusions showing strong pressor effects were also effective in relaxing intestinal strips (rabbit) and in contracting preparations of virgin guinea pig uterus.

While the alkaloid ephedrine has not been isolated from any of our extracts we are led to infer from the above effects that it, or closely related substances, are present.

We failed to obtain uniform results with both the infusions of the crude drug and the solutions of the pure hydrochloride and sulfate. It is of interest to note that the clinical reports to date show similar variations in the human response to this drug.

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[1] Chen, K. K., and Kao, C. H., *J. Am. Pharmacol. Assoc.*, 1926, xv, 625. Miller, T. G., *Am. J. Med. Sci.*, 1925, clxx, No. 2, 157.

* We are indebted to the Cutter Biological Laboratory for this material.

† Expressed in mm. of mercury.

3353

Reciprocal Action of Crop Muscles in Anaphylactic Shock.

P. J. HANZLIK, E. M. BUTT AND A. B. STOCKTON.

From the Department of Pharmacology, Stanford University School of Medicine.

In a previous communication, the demonstration of hypertonicity of circular muscle in the intact crop of anaphylactic pigeons was reported,[1] this change being consistent with muscular hypertonicity in various organs of mammals. However, hypertonicity is not the only functional change in anaphylactic smooth muscle, contrary to usual suppositions. For we have now demonstrated repeatedly a marked hypotonicity of the longitudinal muscle, occurring simultaneously with the marked hypertonicity of the circular muscle in the same organ (crop) during anaphylactic shock in pigeons. There is, in other words, a reciprocal action of these muscles, which occurs also physiologically, but in anaphylactic shock, the action is increased and represents only a quantitative change from normal. The mechanism of the reciprocal action is in the crop itself, because it occurs after section of the autonomic nerves. Epinephrine and atropine act as imperfect antagonists. Our results on nerve degeneration and the myenteric plexuses are as yet indecisive.

The method used permits a simple and easy means of demonstrating and studying the reciprocal action of muscles in the alimentary tract, and also the importance of the functional state in drug and other responses, in the intact organism. The details of the method and of several studies will be published in the near future.

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[1] Hanzlik, P. J., and Stockton, A. B., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 724.

3354

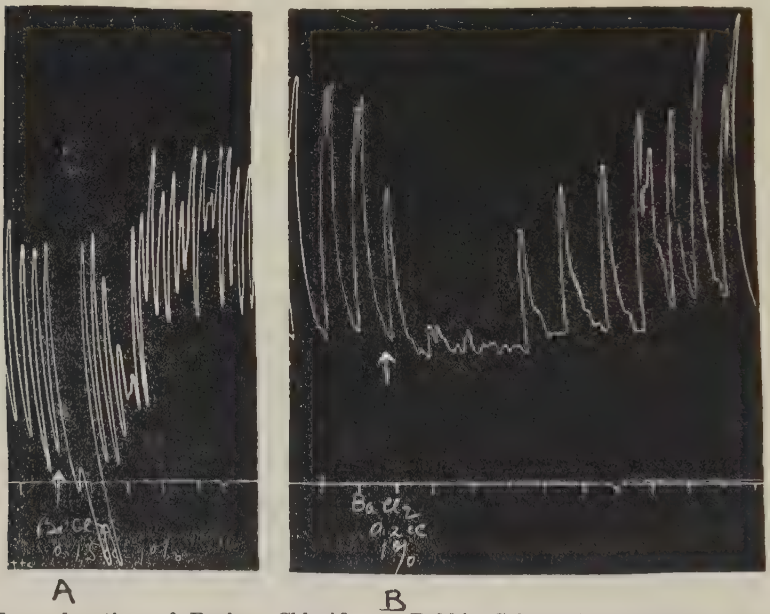
Atypical Action of Barium Chloride on Rabbit Colon.

C. H. THIENES.

From the Department of Pharmacology, University of Oregon Medical School.

During experiments on excised smooth muscle organs, approximately one hundred segments of rabbit colon have been subjected to the action of barium chloride in concentrations of from 1:100,000 to 1:20,000. The segments were suspended in Tyrode solution according to the method of Magnus. Colon segments as a rule were stimulated immediately by the introduction of barium chloride into the bath, resulting in an increase in tonus or in amplitude of rhythmic contractions and often in an increase in rate of contractions. In the case of seven segments, however, the stimulation due to barium chloride was slight, and was preceded by a period of depression, lasting from 15 to 250 seconds and manifested by a fall in tonus and decrease in amplitude. Rate was little affected. The accompanying figure shows two examples of this atypical response to barium.

FIG. 1.



Unusual action of Barium Chloride on Rabbit Colon: A—temporary fall in tonus due to 1:32,500 barium chloride; B—temporary inhibition due to 1:25,000 barium chloride. Time markings represent 30 seconds.

Roth[1] has described primary and persistent depression of rings of the lower intestine of the frog by barium; the writer[2] previously reported barium depression of rabbit uterus suspended in potassium-free Tyrode solution. These depressions agree with the atypical response of rabbit colon. The similarity suggests that barium affects smooth muscle according to the functional state determined by the physical and chemical conditions of its environment.

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[1] Roth, G. B., *J. Lab. Clin. Med.*, 1926, xi, 1149.

[2] Thienes, C. H., *Arch. Internat. Pharmacod. et Therap.*, 1926, xxxi, 447.

3355

Studies in Tuberculosis. X. Skin Tests with Filterable Toxins of the Tubercle Bacillus.

FREDERICK EBERSON AND JESSIE P. DELPRAT.

From the Department of Medicine and George Williams Hooper Foundation for Medical Research, San Francisco.

In a previous report a thermolabile toxin component and thermostable tuberculin element were demonstrated in Berkfeld filtrates of broth cultures of tubercle bacilli.

The present investigation is an experimental clinical test of these observations in a preliminary series of 55 tuberculous patients, and groups of non-tuberculous patients and apparently normal persons as controls.

Berkfeld filtrates were prepared from 26-day-old broth cultures of a moderately virulent human strain of tubercle bacilli incubated at a temperature of from 37° to 39° C. One-tenth cubic centimeter of a 1:20 dilution of filtrate was injected intracutaneously on the anterior aspect of one forearm, and on the other the same dose of filtrate, previously heated at a temperature of 63° to 65° C. for one hour. Controls were made with equivalent amounts of the uninoculated culture media.

Skin reactions were read after 24, 48 and 72 hours, and a note made of the time when the tests became entirely negative. Maximum reactions were observed uniformly after 48 hours, and usually showed definite infiltration and marked erythema at the sites of injection. Control material resulted negatively in all cases. Areas of reaction were approximated by measuring the extent of infiltration and reddening and recording the results in square centimeters.

The observations are summarized as follows:

The unheated Berkfeld filtrate gave the strongest and most persistent reactions as compared with those given by the heated material

Apparently normal persons, and those who had some clinical disease other than tuberculosis, gave sharp reactions of much longer duration and severity than did the tuberculous patients.

Tuberculous patients who could be classified as far advanced (50 cases) and relatively early pulmonary tuberculosis (5 cases) gave reactions which pointed to a clear-cut correlation between the type of skin reaction and the clinical course of the disease.

Analysis of the results in the group comprising 50 patients with far advanced tuberculosis showed that slightly positive skin tests might occur after 24 hours and persist for a day or two, culminating in a ham-colored or brownish pigmentation. At other times there might be a moderately severe reaction which faded away after 24 hours. Such results occurred in a group of 12 patients who were rapidly declining in health, and who had serious tuberculous complications, such as intestinal of laryngeal involvement. In this group 3 deaths have occurred at this writing. The remaining patients of the series gave strong reactions after 24 hours, increased in severity after 48 hours, and persisted for several days. Clinical analysis of the group brought out the fact that these patients were progressing favorably and were showing definite signs of improvement.

TABLE I.
Summary of Results in Group with Far Advanced Tuberculosis.

Area of skin reaction (sq. cm.) after 48 hrs.	No. of Patients.	Condition of Patients	
0-15	12	Extremely ill	9
		Dead	3
15-60	38	Arrested	14
		Improving	24

TABLE II.
Comparative Results of Unheated and Heated Berkfeld Filtrates.

Area of Skin Reaction (sq. cm.) after 48 hrs.	Unheated	Per cent	Heated	Per cent
0-20	33	66	44	88
20-40	10	20	2	4
40-60	7	14	4	8

TABLE III.
Results in Group of Females Among 50 Tuberculous Patients.

Area of Skin Reaction (sq. cm.)	Failing	Type of Reaction			Improv.
		Weak	Moderate	Strong	
0-20	8	5	3		
		0		3	3
20-40	1	0	1		
				1	1
40-60	0				
		0	0	4	4

Deductions from this study warrant the statement that Berkfeld filtrates of broth cultures of tubercle bacilli contain a thermolabile toxin element which gives skin reactions that can be correlated with the clinical progress of a tuberculous infection.

Definitely positive tests appear to be associated with evidence of clinical improvement, despite the presence of a tuberculous condition which might be classified as far advanced.

Skin reactions with Berkfeld filtrates are probably a measure of the resisting capacity of the patient or of normal persons, and as such are indicative of antibody content in the tissues.

The importance of evaluating the clinical course of groups of tuberculous patients must be emphasized in any attempt to interpret the significance of such skin reactions.

Further studies are now in progress with Berkfeld filtrates of tubercle bacillus cultures to determine toxicity and immunizing properties. More extensive series of tuberculous patients and normal persons are being studied along the lines indicated in this progress report.

3356

Thermolabile Toxin and Thermostable Tuberculin Components in Berkfeld Filtrates of Broth Cultures of Tubercle Bacilli.

FREDERICK EBERSON.

From the Department of Medicine and George Williams Hooper Foundation for Medical Research, University of California.

In these experiments, normal and tuberculous guinea pigs were used to determine by intracutaneous tests whether or not Berkfeld filtrates prepared from broth cultures of tubercle bacilli contain toxins which might be distinguishable from tuberculin elements. One series of tuberculous animals had been infected 3 weeks prior to the test and showed early tuberculosis with little, if any, toxemia. The other group of animals, infected 4½ months previously, showed well marked signs of advanced tuberculosis.

A human strain of virulent tubercle bacilli was inoculated into ordinary infusion broth containing 5 per cent glycerine. The Berkfeld filtrates were prepared from a 26-day old culture grown at a temperature of 38° to 40° C. One lot of the filtrate was heated at a temperature of 63° to 65° C. on a water bath. Both portions were preserved with 0.25 ether tricresol. At the time the experiments were made, the material had been stored in the ice-chest for 4 days.

The animals were injected intracutaneously with 0.03 cc. of the heated and unheated filtrates, respectively, in a dilution of 1:2 (with

TABLE I. *Intracutaneous Reactions with Berkfeld Filtrates of Human Tubercle Bacillus.*

Guinea Pigs	Heated Filtrate* (Diluted 1:2)		Unheated Filtrate (Diluted 1:2)		Filtrate and Normal Guinea Pig Serum†		Filtrate and Serum from Tuberculosis Guinea Pig†	
Normal	24 Hrs.	0.2x0.2 cm.; slight infilt. and reddening	24 Hrs.	0.75x0.75 cm.; marked infilt. and reddening	24 Hrs.	Negative	24 Hrs.	0.25x0.25 cm.; slight reddening and slight infilt.
	48 Hrs.	Reaction lessened	48 Hrs.	0.4x0.4 cm.	48 Hrs.	Slight reddening	48 Hrs.	0.2x0.2 cm.; slight reddening
	72 Hrs.	Faint reaction		3rd to 6th day still positive		Negative on 3rd day	72 Hrs.	Slight reddening
		Negative with residual pigmentation, on 4th day		Negative on 7th day				Negative on 5th day
Early Tbc. (21 day inf.)	24 Hrs.	0.5x0.5 cm.; slight reddening and slight infilt.	24 Hrs.	1.0x1.0 cm.; deep infilt., reddening and cent. zone 0.5x0.5 cm.	24 Hrs.	0.25x0.25 cm.; slight reddening	24 Hrs.	0.5x0.75 cm.; reddening and slight infilt. with erythematous border
	48 Hrs.	Reaction lessened					48 Hrs.	0.25x0.25 cm.; reddening and scarring
	72-144 Hrs.	Still positive				Negative on 2nd day		Negative on 4th day
		Negative on 7th day		Strongly positive on 7th day				
Advanced Tbc. (4½ mo. inf. with toxemia)	24 Hrs.	1.0x1.0 cm.; blanching and slight infilt.	24 Hrs.	1.5x1.0 cm.; blanching and erythematous border; central purplish zone; reddening and infilt.	24 Hrs.	1.5x1.0 cm.; edema, reddening, infilt. and cent. purplish zone	24 Hrs.	1.0x1.0 cm.; blanching and edema; cent. purplish zone with marked erythematous border of 2-3 cm.
	48 Hrs.	1.2x1.2 cm.; blanching and slight infilt.	48 Hrs.	1.25x1.25 cm.; deep infilt. and same picture as 24 hrs.	48 Hrs.	1.5x1.5 cm.; as above with deep infilt. and attachment to underlying tissue	48 Hrs.	1.0x1.0 cm.; same as preceding day
	72 Hrs.	1.0x1.0 cm.; slight reddening and slight infilt. with cent. grayish zone	72 Hrs.	1.0x1.0 cm.; same as preceding day	72 Hrs.	1.0x1.0 cm.; very marked reddening, infilt. and cent. grayish zone 0.3x0.3 cm.	72 Hrs.	0.5x0.5 cm.; infilt. and small cent. zone
	7th Day	Slightly positive reaction	7th Day	Reaction is still strongly positive	7th Day	0.5x0.5 cm.; less reddening and infilt.	5th Day	0.5x0.5 cm.; reddening and deep infilt.
		Negative, on 14th day with residual pigmentation		Negative, on 14th day, with residual pigmentation	10th Day	Reaction is still positive	7th Day	0.3x0.4 cm.; reduced infilt. and reddening
						Negative on 17th day	10th Day	Reaction is faintly positive
								Negative on 17th day

*Material was heated on a water bath at temperature of 63-65° C. for 1 hour.

†Berkfeld filtrate was combined with equal volume of serum and incubated 1 hour at 37° C. on water bath, before injection.

physiological salt solution). Another series of experiments was set up with unheated filtrates combined separately with equal volumes of normal guinea pig serum and with serum from tuberculous guinea pigs, and incubated for one hour at 37° C. on a water bath before injection. The object of these experiments was to determine the effect, if any, which normal and tuberculous guinea pig serum might have upon the reacting properties of the filtrates. These combined materials were injected in 0.03 cc. amounts as in the preceding series and controlled with guinea pig serum alone.

The results of the skin tests showed conclusively that unheated Berkfeld filtrates gave more pronounced reactions and those of longer duration than did the heated filtrates, which were often negative. From the chart it can be seen that the guinea pigs with advanced tuberculosis gave in all cases very positive reactions which persisted for as long as from 14 to 16 days. Again, the group of animals with early tuberculosis gave more pronounced reactions, which were of longer duration, than those observed in the normal animals. Experiments with the filtrates which had been combined with normal and tuberculous guinea pig serum indicated that normal serum exerted some neutralizing action on the toxin element, while the serum from tuberculous guinea pigs did not. Finally, the more decided reactions observed in all the groups of animals injected with filtrates which had been previously combined with tuberculous serum, showed that a tuberculin element had been superadded by the tuberculous serum, while excluding the presence of any neutralizing antibody.

These observations indicate that Berkfeld filtrates prepared from broth cultures of tubercle bacilli contain a heat sensitive toxin-like substance which can be distinguished from the tuberculin element present in ordinary tuberculins. Both the toxin and tuberculin components can be demonstrated at the same time in cultures which have grown for from 3 to 4 weeks. By a simple procedure of heating the filtrate at a temperature of from 63° to 65° C. for one hour on a water bath, the toxin is inhibited or destroyed, while the heat-resistant tuberculin is left free to give typical skin reactions. These observations are in agreement with those reported for sera from tuberculous patients and animals.[1]

It remains to be determined at what point during the incubation period of tubercle cultures the tuberculin element first makes its appearance. This substance can be considered doubtless as a disintegration product of the organisms. In order to be certain that subsequent experiments with Berkfeld filtrates from the standpoint of pure toxin production be decisive, it will be necessary to establish this point. Obviously there must be a time when toxin production is at a maximum and when the progressive developmental cycle of thermolabile and thermostable components can be studied. To this end experiments are now being made with different strains of tubercle bacilli grown in different media for varying periods of time. Work now in progress with a rapidly growing strain of tubercle bacilli points to the presence of a tuberculin-free thermolabile component which gives skin reactions in normal and tuberculous patients and animals. These results can be correlated not only with the stage of infection but with the clinical progress of the disease. There is clear-cut evidence also that most of the patients with far advanced tuberculosis do not give minimal or negative tests unless antibodies are absent, contrary to the report of Herrold and Saelhof.[2]

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[1] Eberson, F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 79.

[2] Herrold, R. D., and Saelhof, C. C., *J. Am. Med. Assn.*, 1926, lxxxvi, 747.

3357

A Note on Tissue Respiration in Relation to Thyroidectomy.

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From the Division of Biochemistry and Pharmacology, University of California Medical School.

In the course of a study of the influence of lactic acid upon tissue respiration it was observed that tissues from thyroidless rats showed a lower rate of oxygen consumption than did similar tissues from normal rats.

Bits of diaphragm tissue suspended in Ringer solution containing 0.2 per cent glucose and buffered to pH 7.5 by a M/80 phosphate mixture under an atmosphere of oxygen were studied by Warburg's[1] manometric method.

Normal.		Thyroidless	
Wt. of animal.	Q_{O_2}* 1st hour.	Wt. of animal.	Q_{O_2}* 1st hour.
gm.		gm.	
142	8.2	174	4.0
145	8.8	175	5.3
172	6.4	241	5.3
175	9.6	177	6.8
180	5.7	155	4.5
mean	7.7	mean	5.2

* Q_{O_2} = cubic millimeters O_2 per mg. dry tissue per hour.

The results are of interest in connection with the recent paper of Grafe, Reinwein and Singer,[2] who found that the rates of respiration of surviving tissues of animals varying in size from the mouse to the ox, are of substantially the same magnitude, whereas the oxygen absorption per kilogram of the whole living animal is some 20 times greater for the mouse than for the ox, thus demonstrating that the constant relation between metabolism and surface area is a function of the intact animal, and not a result of inherent characteristics of the protoplasm. These authors seek an explanation in considerations of oxygen supply to the tissues and the relative number of patent capillaries. In the case of our thyroidless rats the metabolism of the living animal is 25 to 30 per cent below the average normal, as is also the respiration of its isolated tissues under conditions of maximum oxygen supply.

My thanks are due to Professor P. E. Smith, who gave me the thyroidectomized animals. Further work is in progress.

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[1] Warburg, O., *Biochem. Z.*, 1923, cxlii, 317.

[2] Grafe, E., Reinwein, H., and Singer, *Biochem. Z.*, 1925, clxv, 102.

3358

Influence of Age on Degree of Renal Hypertrophy Produced by High Protein Diets.*

LOIS LOCKARD MAC KAY, EATON M. MAC KAY AND T. ADDIS.

From Stanford University Medical School.

Three adequate diets which contained 18 per cent, 31 per cent and 70 per cent of protein in the form of casein, which differed only in respect to a replacement of cornstarch and lard by casein, were fed to three groups of male albino rats from the time they were 26 days old until they were 70 days of age. The animals were then killed and the kidney weights ascertained. Table I shows that as the amount of protein increased there was a considerable increase in the weight of the kidney.

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*This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

TABLE I.
Protein Intake and Kidney Weight in Young Rats.

Protein eaten per rat per day.	Average kidney weight.	Average body weight.	Number of animals.
gm.	mg.	gm.	
2.52	646	159	25
4.39	835	199	18
10.44	963	149	29

The same diets were also given to adult rats from the time they were 346 days of age until they were 400 days old. Table II shows that the increase in the consumption of protein had little effect on the weight of the kidney in these older rats.

TABLE II.
Protein Intake and Kidney Weight in Adult Rats.

Protein eaten per rat per day.	Average kidney weight.	Average body weight.	Number of animals.
gm.	mg.	gm.	
2.14	1034	351	22
4.10	1102	398	13
7.13	1145	299	26

3359

Do High Protein Diets Increase Weight of Kidney Because They Increase Nitrogen Excretion?*

LOIS LOCKARD MAC KAY, EATON M. MAC KAY AND T. ADDIS.

From Stanford University Medical School.

The experiments cited in the preceding note[1] served as controls for experiments in which the same order of magnitude of increase in nitrogen consumption was obtained by the administration of urea instead of protein. The results given in Table I suggest that the simple hypothesis of a work hypertrophy may be inadequate as an explanation for the increased kidney weight in young rats, and demonstrate that in adult rats a marked increase in nitrogen consumption may have no effect on the weight of the kidney.

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* This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

TABLE I.
The effect on kidney weight in young and adult rats of increase in nitrogen consumption derived from addition of protein or from addition of urea.

Diet.	Young Rats.		Adult Rats.	
	Nitrogen eaten per rat per day.	Average kidney weight.	Nitrogen eaten per rat per day.	Average kidney weight.
	mg.	mg.	mg.	mg.
18% protein	402	646	344	1034
31% protein	700	835	656	1102
Urea + 18% protein	746	729	467	1033
70% protein	1680	963	1140	1145
Urea + 18% protein	1810	838	858	1035

[1] MacKay, L. L., MacKay, E. M., and Addis, T., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 335.

3360

Genital System Responses to Daily, Pituitary Transplants.

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From the Department of Anatomy, Stanford University.

The daily transplantation of pituitary tissue from full-grown rats into immature female rats hastens development.[1] Daily, pituitary homeotransplants, the donors being adults, likewise hastens the growth of the genital system in the immature male rat. Nine animals from 4 litters have been treated, each having 1 or more littermate control. Results gained from 1 of these litters illustrate the typical response to the transplants.

Protocol. Of a litter of 4 males daily pituitary homeotransplants were begun in 2 on the 14th day of life, 2 serving as controls. The animals were nearly identical in weight.

One treated and 1 control were autopsied 10 days later (age 24 days). *Treated animals:* Weight, 44 gm.; weight of both testes, 0.254 gm.; of genital system exclusive of testes, 0.480 gm. *Control:* Weight, 43 gm.; weight of testes, 0.240 gm.; of genital system exclusive of testes, 0.249 gm.

The remaining 2 animals were autopsied at the age of 31 days, the treated animals thus having received 17 transplants. *Treated* *animal:* Weight, 74 gm.; weight of both testes, 0.603 gm.; of genital system exclusive of testes, 0.916 gm. *Control:* Weight, 69 gm.; weight of both testes, 0.339 gm.; of genital system exclusive of testes, 0.409 gm. (Weight of the genital system include the empty urinary bladder.)

It is seen that there is no marked effect upon the size of the testes in the younger animal from these transplants. The genital system, exclusive of the testes, however, shows a marked increase in weight, transcending all normal variability. It is nearly double normal weight. The other (older) animal shows that with a more prolonged treatment, and possibly also due to an increase in age, the testicular response also becomes marked. The disproportion between the weights of the rest of the genital system of the treated and control animals is likewise increased.

After ablation of the gonads in the immature male, pituitary transplants have no effect upon the remaining components of the genital system. This is identical with results obtained in the immature female.

I have also made daily transplantations of the pituitaries from adult guinea pigs into immature male rats. The technique was identical with that employed in the homeotransplants. These transplantations made in 2 animals for 10 and 12 days, respectively, failed to stimulate the growth of any part of the genital system.

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[1] Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 131.

Massachusetts Branch.

Harvard University, December 15, 1926.

3361

Temperature Characteristics for Heart Rate in Embryos of Limulus.

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From the Laboratory of General Physiology, Harvard University, Cambridge, Mass.

For a number of *Limulus* embryos the relationship has been determined between temperature and the frequency of cardiac contractions, during stages of development when the (myogenic) heart is still devoid of a nervous system. A thermostat maintaining any desired temperature between 0.05° and 45° to within ±0.01° or better, and quickly adjustable, was devised to facilitate this work. The temperature characteristics obtained are not the same in all individuals. The values $\mu = 11{,}500$ and 16,400 are usual; but $\mu = 20{,}000$ and $\mu = 25{,}100$ also occur. These are well recognized values in numerous other vital processes, but differ from the magnitude ($\mu = 12{,}200$) which seems typical for the thermal control of frequency of contraction in the heart of the adult (the temperature of the ganglion being altered). This need not be interpreted as corresponding to an essential difference between myogenic and neurogenic rhythm. The critical temperatures for heart rhythm also differ among individuals; the following are of definite occurrence, in different cases: 9°, 20°, 27°, 31°, 41°, 45°.

The results are chiefly important as indicating relatively greater metabolic lability in embryonic than in adult tissues. It is concluded that the apparent inconstancy of μ for contraction rate in explants of chick myocardium (H. Murray) is due to the presence of several functional pacemakers having different temperature characteristics, corresponding to the finding of different μ's for the hearts of similar *Limulus* embryos. This is proved by the fact that in cases giving atypical μ's, the latitude of variation is not a constant fraction of the mean; the marginal heart-beat frequencies yield typical μ's.

It cannot be held that temperature characteristics are determined by the organism as a whole. The movements of heart and of gills, in *Limulus* embryos, show different μ values in the same individual at the same time; and the critical temperatures for the two activities do not coincide. This is also true in *Asellus*. The critical increments or temperature characteristics have, therefore, a specific, local significance.

3362

Measurements of Fatal Doses of Chloroform in the Brains of White Rats.

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From the Biological Laboratories of Clark University.

Qualitative tests. The aqueous extracts of the brains of frogs, mice, rats, guinea pigs, cats, and dogs, killed by inhaling chloroform, have all shown the presence of chloroform by the pyridine test,[1] while similar extracts from animals killed by electricity, illuminating gas, CO_2, blow on the head, and beheading, have all been negative for chloroform. In a series of 8 brains from rats killed by inhaling chloroform, the tests were made at the end of 1, 1½, 2, 3, 5, 6, 15 and 25 days respectively. In each case chloroform was indicated, in spite of the fact that considerable decay of the tissues had occurred. These results agree with those reported by Luedeking,[2] and Angiolani,[3] to the effect that chloroform in animal tissues persists unchanged for many days after death and decay.

Quantitative tests. The chloroform present in extracts of brains from rats killed by inhaling chloroform has been measured by the pyridine colorimetric method. Into a 1-liter dish containing a rat, a known amount of chloroform was sprayed. At the moment of death (cessation of respiratory movements) the rat was removed and its brain extracted. A dose of 0.1 cc. was sub-lethal in 35 minutes; 0.2 cc. caused death in an average of 11.2 minutes; 0.4 cc. in 5.4 minutes; 0.8 cc. in 4.3 minutes; 1.0 cc. in 3.5 minutes; 2.0 cc. in 2.5 minutes; 4.0 cc. in 1.6 minutes and 5.0 cc. or more in 1.5 minutes. The time of death bore no clear relationship to size of the animal. In Table I are given the amounts of chloroform found in brains of killed and anesthetized rats.

TABLE I.

The amounts of chloroform expressed as cc. per milligram of brain tissue (fresh), in rat brains following death and anesthesia by the inhalation of chloroform vapor.

Dosage: cc. in 1-liter dish.	Chloroform in brain tissue, cc. per mgm.
0.2	0.03780
0.4	0.03579
0.8	0.06810
1.0	0.07281
2.0	0.09136
4.0	0.08992
5.0 or more	0.09210
anesthesia, slight	0.01190
anesthesia, deep	0.03028

These results indicate that the amount of chloroform present in the brains of rats at the time of death varies directly with the dosage. In other words, the amount of chloroform in the brains of rats killed by chloroform is not constant. This fact may be explained by assuming that the immediate cause of death from chloroform is exerted on some tissue or organ other than the brain; or that the effect of chloroform on the brain is secondary in so far as death is concerned. Further experiments are being done to identify the tissue or organ whose poisoning by chloroform causes death.

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[1] Cole, W. H., *J. Biol. Chem.*, 1926, lxxi, 173.

[2] Luedeking, C., *Am. Chem. J.*, 1886, viii, 358.

[3] Angiolani, P., *Chem. Centralbl.*, 1891, lxii, 1068.

3363

Galvanotropism of Roots.

A. E. NAVEZ. (Introduced by W. J. Crozier.)

From the Laboratory of General Physiology, Harvard University, Cambridge, Massachusetts.

Every one interested in plant irritability has always considered the galvanotropic response of roots as remarkable.

From previous work it could be deducted that for high densities of current or long exposures, anodic curvature (Elfving's curvature) is obtained.

For low densities of current or short exposure, cathodic curvature (genuine galvanotropic response) is obtained.

The opinions differ in many respects regarding the origin ascribed to this last curvature. Brunchorst believes an injury is produced by H_2O_2; Rischawi considers the phenomenon similar to the electrosmosis of water in du Bois-Reymond's experiments with albumin. Ewart and Bayliss attribute this fact to chemiotropic stimulation. Gassner finds it a traumatic response to unilateral injury.

But in practically all this work the technic is too crude. For instance, the formation or diffusion of products of electrolysis is scarcely considered; often electrodes and roots dip in the same liquid. Often, too, the density of current is not given.

For these reasons the experiments were repeated and the technic elaborated keeping in mind the following points: (1), reduction of polarisation products (non-polarizable electrodes); (2), prevention of diffusion of electrolysis products by agar blocks; (3), variable densities of current by use of troughs with definite geometrical shapes and sizes.

The electrodes used were zinc—zinc sulphate electrodes. The densities of current varied between 0.058 MA/cm^2 and 1 MA/cm^2, the potential difference from 0.5 volt to 115 volts; the time of exposure from 15 minutes to 360 minutes. The plants used were *Vicia faba* and *Phaseolus vulgaris*. The seedlings, grown in sawdust at 20° C., were employed when the roots had 4 to 6 cm. in length, the part dipping in the liquid ranging from 8 to 10 mm.

When all products of electrolysis are prevented from reaching the roots (*i. e.,* trough with agar blocks and non-polarisable electrodes) no curvature is shown. In a trough without the agar blocks, put in series with the first, all the roots curve perfectly.

The immediate conclusion is that the products of electrolysis act directly to produce "galvanotropic" curvature.

The current itself is not indispensable to cause a curvature. If the roots are placed in a trough without agar blocks, where undisturbed electrolysis products have been formed, these roots curve, but in an indeterminate way. The current determines therefore the direction of curvature.

That the electrolysis products act as injuring agent on the root is demonstrated by the following experiment: Roots are placed for 2 minutes in a $N/_{100}$ solution of copper nitrate, and subjected thereafter to a current between agar blocks and non-polarisable electrodes; curvature occurs.

The experiments show that two steps must be considered: (1) the toxic effect of electrolysis products; (2) the further increase of the first by persistence of the current.

If we consider a root, circular in cross section, we can assume that by the toxic action of the electrolysis products a larger number of cells on the two opposite halves of the root are injured. These cells may act now as two electrodes directly applied on the internal tissues.

These electrodes determine an electrical field where the conducting paths are made by the cellulose membranes imbibed with aqueous solutions (the protoplasm itself is, practically, a perfect dielectric for D. C.). The resulting electrolysis acts now on the deeply situated cells.

From this action there results inside of each cell chains of + and — charges, and consequently a relative increase of ions on each side of the root (increase of anions on the anodic side, of cations on the cathodic side).

The first effects of the anions is not to prevent growth. On the contrary, the increase of cations slows the growth, and by further increase stops it completely. The total effect is a bending towards the cathode.*

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* Full account will be found in *J. Gen. Physiol.*, x, in press.

Illinois Branch.

(With Society of Internal Medicine.)

City Club, Chicago, Ill., December 20, 1926.

3364

The Influence of Malaria Chills on the Trypanocidal Action of the Serum.

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From the Department of Pathology, College of Medicine, University of Illinois, and the Uihlein Memorial Laboratory of the Grant Hospital of Chicago.

During their experiments on the natural immunity of man against the trypanosomes, which are pathogenic to animals, Laveran and Mesnil[1] discovered the trypanocidal action of the human serum. The serum does not destroy the trypanosomes *in vitro,* but it protects mice for from 1 to 2 weeks against an infection, which otherwise would cause death within a few days. It delays, therefore, only the development of the trypanosomes in the mouse. A complete protection is rare.

The trypanocidal substances are scanty in or absent from the serum of the newborn (Rosenthal and Nossen,[2] Platau,[3] Leichtentritt,[4] Neumark and Pogorschelsky[5]). In healthy children they appear after the 11th to 15th week and persist throughout life, although individual differences as to their strength exist. According to Rosenthal and Kleeman[6] the trypanocidal substances are increased in the later part of pregnancy. Under certain abnormal conditions they disappear more or less completely. Leichtentritt and Zielaskowsky[7] found a marked decrease in Barlow's disease, Grunwald and Leichtentritt[8] in xerophthalmia and infantile scurvy. Much attention has been given to the fact that diffuse diseases of the liver cause a very distinct reduction of the trypanocidal power of the serum (Ehrlich and Wechsberg,[9] Rosenthal and Krueger,[10] Mignoli,[11] Peutz,[12] Platau,[3] Munter[13]). Since the accumulation of the bile pigment and the bile acids in the blood has nothing to do with this reduction, the liver is believed to be the source of the trypanocidal substances. Several authors tried to use the trypanocidal titer of the serum as a liver function test.

Plaut[14] studied the trypanocidal action of the serum in various mental diseases and observed that highly active sera were very common among patients with general paralysis. Neumark and Pogorschelsky[5] found an early appearance of the substance under discussion in congenital syphilis.

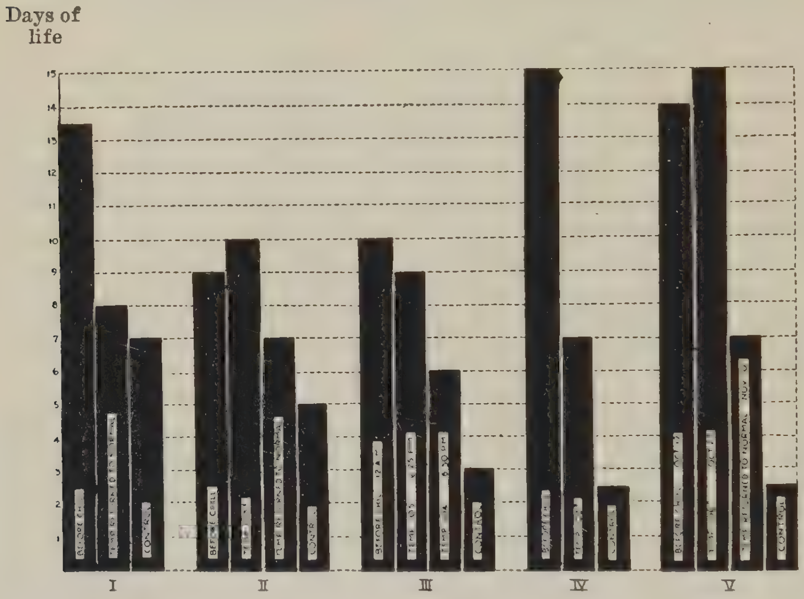
Whether infections affect the trypanocidal titer of the serum has not yet been definitely determined. Leichtentritt[4] did not see any influence especially in mild infections of early childhood. Mignoli[11] saw no changes in septicemia, but a diminution in cases of severe tuberculosis. Neumark and Pogorschelsky[5] observed a decrease in infectious diseases with fever. Barlowi[15] described a low titer during infections and a restoration to normal during convalescence.

The use of malaria treatment in general paralysis offered the possibility of following more closely the effect of the malaria chills on trypanocidy of the serum.

A strain of *Trypanosoma equiperdum* obtained through the courtesy of Dr. Taliaferro was used. The intraperitoneal injection of 0.1 cc. of a suspension containing about one trypanosome in a high power field killed mice, 17 to 20 gm. in weight in from 2 to 5 days. The sera were used within 24 hours after they were drawn and were injected subcutaneously. In preliminary experiments with sera of normal individuals, and of patients suffering from various diseases except those of the liver, it was found that the average length of protection produced by 0.1 or 0.2 cc. serum was 11 days. Seven per cent of the mice were completely protected. In the beginning 0.05, 0.1, 0.15 and 0.2 cc. of serum were injected. In these experiments paradoxical results were sometimes obtained, in so far as smaller doses protected more than larger ones. Similar observations were made by previous investigators. There exist apparently individual differences as to the susceptibility of mice to the trypanosome infection. These differences are revealed only by the simultaneous injection of the protecting human serum. Sometimes trypanosomes appeared in the serum mice at the same time as they did in the untreated controls. But they disappeared after a few days, to come back and to increase rapidly in number until the animal died.

To overcome these complications only two doses of serum, namely 0.1 and 0.2, were finally given. Six mice were used in each series. The blood was tested daily for trypanosomes. The average length of life after the infection of all the mice of each series was used as trypanocidal titer.

The frequent occurrence of highly protecting sera in cases of general paralysis mentioned by Plaut[14] was also seen in our experiments. Especially complete protection of mice was more common than in the other cases, namely 20 per cent against 7 per cent.



In case I blood was drawn immediately before and after the third chill. The highest temperature during this chill was 105^6. A very mild infection was used as shown by the long survival of the controls.

In case II the samples were taken 2 hours before the 8th chill, 4 hours after the temperature had reached the peak which was 106, and 2 hours after the temperature had returned to normal.

In case III the blood samples were obtained during the 5th chill. The highest temperature in this chill was reached at 5:30 p. m. and was 105^4.

In case IV blood was drawn 2 hours before the 6th chill and 6 hours after the peak of the temperature rise (105^6).

In case V the first sample was taken a few hours before the first chill started. The second sample was obtained at the peak of the 4th chill, and the last sample was secured the morning after the 16th chill.

These experiments show that the malaria chills cause a marked reduction of the trypanocidal titer of the serum. At the height of the fever there may be a slight increase of the titer, to be followed soon by a sharp drop. This drop is most pronounced shortly after the temperature has reached the normal level and is transient. The normal power of the serum is restored before the next chill starts. It makes no difference during which chill the blood samples are taken, since in each chill the same changes take place.

We do not know whether the liver cells during the malaria chills are injured. If the liver is really the source of the trypanocidal substances, their disappearance after the chills may point towards such a possibility. Other immune substances like the opsonins increase with each malaria chill (Hoff and Silberstein[16]).

Conclusions: During the malaria chills the trypanocidal action of the serum is distinctly diminished. This decrease is most pronounced shortly after the temperature has returned to normal. Before the next chill starts the normal titer is restored.

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3365

Histological and Microchemical Studies on the Lipin Content of the Human Thyroid.

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From the Department of Pathology, College of Medicine, University of Illinois.

The lipoid droplets found in the epithelial cells of normal human thyroids are the products of the secretory activity of these cells. According to their staining reactions they seem to consist mainly of mixtures of phospholipins. They are discharged into the colloid where they become gradually dissolved.

The cells excrete the lipoids in the form of small droplets and granules which are suspended in larger drops of a watery substance that does not take the fat stains. The fat droplets are soluble in ether, alcohol and acetone, but insoluble in chloroform. They stain blue or bluish violet with nieblue sulfate, and black with the Lorrain Smith method. Some of them are stained after the Caccio's method. In older persons also clusters of larger fat drops are given off by the epithelium. These drops dissolve only in hot ether and are usually Caccio positive. A desquamation of cells filled with fat is relatively rare.

The excretion of the lipins starts after the first year of life and increases with the age. But also in younger individuals much fat is occasionally found in the colloid. What influences the intensity of the lipoid excretion is not yet quite understood. Diseases seem to have no effect.

In pronounced exophthalmic goiter, in tuberculosis of the thyroid, and in malignant adenomas fat is usually absent from the epithelium and the colloid.

Lipoid granules are also observed in the stroma of the thyroid. They give the same microchemical reactions as do the fatty substances in the epithelium and colloid. They are engulfed by flat and branched cells.

An interesting finding is the distinct staining of the plasma in the smaller blood vessels with sudan III. It was found in 45 per cent of the thyroids examined. This local lipemia not seldom is so intensive that it resembles the changes seen in lipemic diabetes or in experimental hyercholesterenimia of herbivorous animals.

The microscopical pictures under discussion were not only observed in thyroids secured from autopsies, but also in glands removed during life, in plain goiters as well as in normal specimens.

This excludes the possibility of dealing with post mortem or degenerative changes.

3366

A Specific Carbohydrate from Bacterium Enteritidis.

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From the Department of Hygiene and Bacteriology, The University of Chicago.

During recent experiments with the soluble antigens of *Bacterium enteritidis,* large quantities of toxic Berkefeld filtrates of cultures grown in a simple salt medium were evaporated to dryness *in vacuo,* and this dry residue subsequently dialyzed. During the dialysis a grayish white precipitate settled out of the material in the dialyzing sac. This precipitate gave none of the usual protein tests; *i. e.,* biuret, Millon, vanillin, diazo, or ninhydrin; but it gave a brilliant Molisch test, indicating that it contained carbohydrate. Saline extracts of this material gave precipitin reactions with *B. enteritidis* antiserum, and not with any other serum tested.

Sixty per cent, by weight, of the material in this precipitate proved to be inorganic, and was chiefly diatomaceous earth from the Berkfeld filters used; 40 per cent was organic matter.

The carbohydrate was separated from the diatomaceous earth with difficulty. Either it is very slightly soluble in ordinary solvents, or it is adsorbed by the diatomaceous earth. The most satisfactory method tried so far has been the following: The dry carbohydrate-containing material was heated for 1 1/2 hours at 150° C. A weighed amount was placed in a small flask, distilled water added, and the suspension autoclaved at 5 pounds pressure for 3 hours. The diatomaceous earth was then removed by centrifugalization, and the supernatant fluid evaporated to dryness on a water bath. The small white residue adheres to the dish and does not redissolve readily, but it is sufficiently soluble to give a vivid Molisch test and a specific precipitin reaction with *Bacterium enteritidis* antiserum. In this way 77 per cent, by weight, of the organic matter was extracted from the diatomaceous earth mixture.

The usual method of hydrolyzing starch with N/2 HCl failed to yield any trace of sugars that reduce Fehling's solution. The carbohydrate was apparently unchanged by this acid treatment, for when the solution was evaporated to dryness on a water bath the residue still gave a specific precipitin test with *Bacterium enteritidis* antiserum.

The carbohydrate seems to be slightly dialyzable. An alkaline extract giving good Molisch and precipitin reactions was placed in a small collodion sac and dialyzed with a constant volume of distilled water. At the end of 48 hours the water outside the sac gave a faint Molisch test; the material inside the sac gave a much stronger test.

The evidence so far obtained seems to indicate that this carbohydrate obtained from *Bacterium enteritidis* is a stable, difficultly soluble polysaccharide. Its claim for specificity rests upon the fact that solutions containing it have been found consistently to give specific precipitin reactions with *Bacterium enteritidis* antisera, and that the intensity of this precipitin reaction seems to be correlated with the concentration of the carbohydrate in the solution. There was no protein demonstrable in this material, but the possibility that an undetectable amount might serve as an antigen for precipitation tests must not be ignored. There was not enough material left for a quantitative micro-nitrogen determination. Since the culture medium used contained an ammonium salt, such a procedure would have been of doubtful value.

So far, too little of this substance has been available to investigate its ability to stimulate specific antibody formation in animals. Preparation of material for further study is now in progress.

3367

Parathyroid Hypercalcemia and Anaphylactic Shock.

S. A. LEVINSON AND S. A. MATHEWS.

From the Department of Pathology, University of Illinois, College of Medicine, Chicago, and the Department of Pharmacology, Loyola University.

It has been suggested that an increase in blood calcium might diminish anaphylactic shock, and that the hypercalcemia following the injection of parathyroid hormone might be useful in this connection.

To study this point a series of experiments have been carried out in dogs which had been sensitized to egg albumin.

Of 8 sensitized animals, 4 received 5 cc. of the Collip Para-thormone on the day preceding the injection of the shocking dose. Calcium determinations made on these animals before the reinjection of the egg albumin showed calcium levels from 12.6 to 15 mg. per

100 cc. of serum, with corresponding low K/Ca ratios. The pulse rate was low (42-60), but the blood pressure within normal limits.

On intravenous injection of the antigen no appreciable difference was noted in the response of the animals with hypercalcemia, and those not treated with the parathyroid extract. The duration and degree of fall in blood pressure, as well as the other manifestations of the shock, (leukocyte count, coagulability, gastro-intestinal effects and temperature changes) were practically identical.

3368

Capillary Permeability During Pulmonary Tuberculosis.

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From the Laboratory of the Municipal Tuberculosis Sanitarium, Chicago, and the Department of Pathology, University of Illinois, College of Medicine.

In a paper previously published[1] a method has been described whereby clinical variability in capillary permeability has been examined by means of the blister (estimated by per cent of protein exudated, and by duration of time required for the blister to form).

In the present study we have examined such cantharides blisters produced on 250 tuberculous patients.

It has been found that there is a progressive increase in the degree of permeability with active progression of the disease, illustrated in the following table.

Subjects	No. in Group.	Ave. Permeability Ratio.	% Below Ave.
Normal students	66	68	50%
Students with evidences of more than the usual amount of healed tuberculous lesions	24		58%

1. A and B cases (N. T. A. Classification)	9	55 per cent
M. A. A. Cases	33	42 per cent
M. A. B. Cases	11	43 per cent
F. A. A. Cases	15	33 per cent
F. A. B. Cases	29	17 per cent
F. A. C. Cases	9	22 per cent

When the blister time was examined it has been found that for the normal, as well as for the I A, I B, and the M. A. A. cases, the average was about 7 hours. The M. A. B. cases showed a considerable prolongation (9 hours); with progression of the disease the blister time was progressively shortened.

The changes in the blister time would indicate a moderate increase in sympathetic tonus of the skin during the M. A. A. stages which, however, gives way to a parasympathetic overbalance with progress of the disease. There is evidently a distinct increase in capillary permeability with increased activity of the tuberculous process. Exudative cases in general have capillaries that are considerably more permeable than the normal.

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3369

The Precipitin Content on the Protein Fractions of Immune Serum.

HILDA H. KROEGER AND LUDVIG HEKTOEN.
(Introduced by William H. Welker.)

From the Laboratory of Physiological Chemistry, University of Illinois College of Medicine and the John McCormick Institute for Infectious Diseases.

Rabbits were immunized against crystallized egg albumin. The antiserum produced was separated into the protein fractions, euglobulin, pseudoglobulin, and albumin by 33 per cent, 46 per cent, 64 per cent, and 100 per cent saturation with $(NH_4)_2SO_4$. A precipitin test by the contact method was made on the whole serum and on each protein fraction, in an effort to discover with which fraction the precipitin came down. In all 10 cases the whole serum gave a positive test, with precipitin titers ranging from 100,000 in 2 cases to 10,000,000 in 1 case. The precipitin was carried down with the euglobulin fraction, but during dialysis the pseudoglobulin was split off by hydrolysis and the precipitin remained in this water soluble fraction. The albumin fractions were found free of precipitins in all cases. The precipitin titer was less in the globulin fraction containing the precipitin than in the whole serum, calculated on the basis of equivalent globulin content.

3370

Effect of Visual Impulses Upon Progression and Righting of the Crayfish.

T. KOPPANYI AND N. KLEITMAN.

From the Department of Physiology of the University of Chicago.

In the course of our systematic study of body righting in the crayfish, *Cambarus virilis, Hagen,* we observed the following phenomena. Extirpation of both eyes, including the eyestalks, causes the animals to lose permanently their ability to walk forward. A normal crayfish occasionally walks backward, but its usual mode of progression on land is forward. In a large number of crayfish operated upon we never observed any forward movement after the operation. A similar effect may be produced temporarily by merely blindfolding the animal.

Immediately after removal of the eyes the animals cannot turn over when placed on their back, but after a few days some of the operated animals regain their ability to right themselves from the dorsal position.

3371

Sex Differences in the Contraction Rate of the Human Gall Bladder.

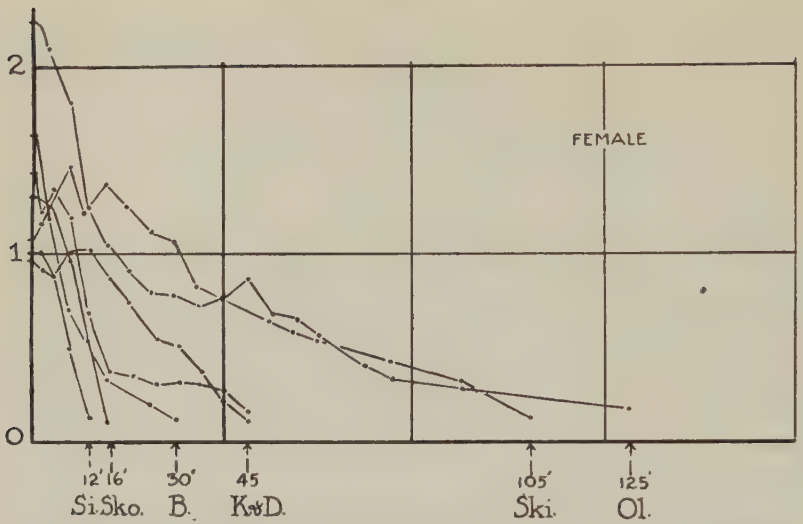
EDWARD A. BOYDEN.

From the Department of Anatomy, University of Illinois College of Medicine, Chicago.

It is generally recognized by practitioners that certain disturbances of the biliary tract are more prevalent among women than among men, especially stout women. The reason for this difference has never been ascertained. Indeed, before the advent of the Graham method no adequate means existed of comparing either the size of this organ or its behavior in the two sexes. In the hope of finding some anatomical or physiological basis for clinical differences the writer has recently begun a study of the contraction of the gall bladder in healthy young women, following a standard meal of 5 egg-yolks and half a pint of cream.

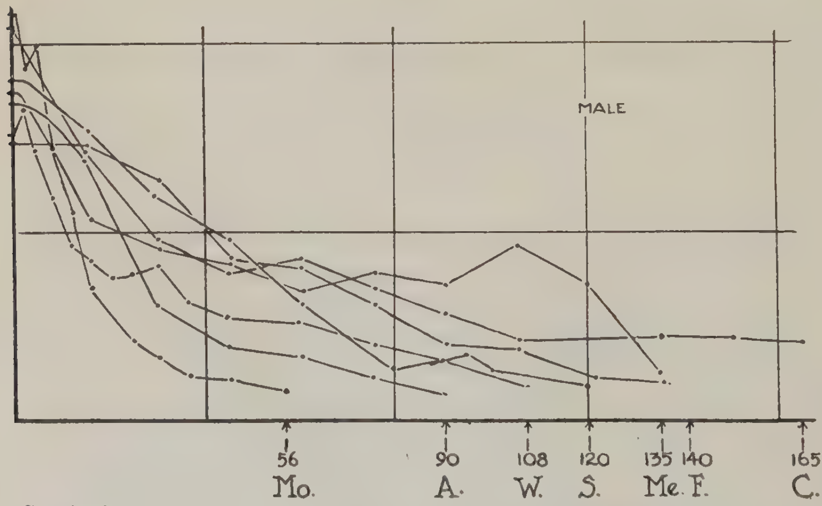
In 7 of these 11 cases it was possible to follow the contraction of the gall bladder until the shadow became very small. The changing

FIG. 1.



Graphs illustrating contraction of gall bladder in 7 young women following a meal of 5 egg-yolks and half a pint of cream. Volumes computed from cholecystograms.[1] *Abscissas* represent time in minutes (40 minutes to a square); *ordinates,* cubic inches of bile. 12', 16', etc., number of minutes required for the gall bladder to reach a volume of 0.18 cu. in. (3 cc.). *Letters* refer to names in Table I A.

FIG. 2.



Graphs illustrating contraction of gall bladder in 7 young men (Table I B), following a meal of 5 egg-yolks and half a pint of cream. 56', 90', etc, number of minutes required for the gall bladder to reach a volume of 0.18 cu. in. (3 cc.). *Letters* refer to names in Table I B.

volume of each series was then computed (Table I A) and contraction curves plotted. As these 7 young women were medical students or laboratory assistants, they belong to the same occupational group, therefore, as the young men previously studied.* A comparison of the two groups (Figs. 1 and 2) shows that the female gall bladder, instead of being more sluggish as might well have been predicted, empties faster than the male, 5 out of 7 cases reaching a very low volume of 3 cc. (0.18 cu. in.) in from 12 to 45 minutes after a meal; whereas 6 of the male gall bladders required over 90 minutes to reach the same low point.

This striking contrast is due neither to a differing latent period (the initial contraction in either sex occurring anywhere from 2 to 16 minutes after food enters the mouth), nor to the fact that the gall bladder of the male is usually larger and therefore has more bile to be expelled. For a comparison of the minimum time required in each sex for the discharge of 1 cu. in. of bile (16.4 cc.), shows that the female gall bladder is nearly twice as quick as the male, the average ratio in these 14 cases being 23:40 (Table I C). Equally instructive is a comparison of the total volume of bile discharged in the two sexes at the end of the first 15, 30, and 45 minute periods, the comparison again favoring the female gall bladder. One may, therefore, infer, provided these figures are supported by a larger number of cases, that the initial digestion and absorption of such foods as egg-yolk and cream take place more rapidly in young women than in young men.

Another significant feature in this comparison of the two sexes is the difference in size of the distended gall bladders per unit weight. Thus in Table II, it is shown that there is 1 cc. of bladder bile for every 3 1/3 pounds of body weight in the young men, but only 1 cc. to every 4 3/4 pounds in the young women. It would thus appear that in respect to size, the female gall bladder is at a disadvantage, especially if toward middle age the body increases greatly in weight, as in fleshy women. This may be offset, perhaps, by the quicker emptying time, so long as that function is unimpaired. An especially interesting case, from this standpoint, is the one shown at *C* in figure 4. In this individual the small gall bladder empties in the first 12 minutes after a meal, partially refills in the next 48 minutes, and then empties again within 40 minutes.

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* Altogether, eight male series with egg-yolk and cream as a diet, have been completed by the writer. One of the eight (H. M. T.) has purposely been omitted from this paper since the gall bladder of this individual is much larger than the others and requires 4½ hours to empty.[1] The remaining seven (Fig. 2. Table I B) form a homogeneous group.

TABLE I.

Discharge of bile from human gall bladder after a meal of egg-yolk and cream.

A. In seven young women.

Name	Age	Wt.	Vol. G.B. before meal	Total discharge of bile			Minimum time required to discharge	
				15'pc.	30'	45'	1 cu. in. (16.4 cc.)	All but 3 cc.
		lbs.	cu. in.	cu. in	cu. in.	cu. in	min.	min.
C. L. B.	29	115	1.66	1.31	1.50	1.50	9.5	30
F. E. D.	18	120	1.03	.16	0.53	0.86	44.	45
R. Sko	34	121	1.30	1.19	1.22	1.22	9.5	16
S. M. O.	24	122	2.21	1.15	1.43	1.49	10.5	125
C. H. Si	29	135	1.26	1.06	1.13	1.16	12.	14
H.H. Ski	28	155	1.47	.09	0.40	0.75	64.	105
H. H. K.	23	190	1.44	1.05	1.11	1.26	12.	45
Totals	185	958	10.37	6.01 (98.6 cc.)	7.32 (120 cc.)	8.24 (135.1 cc.)	160.5	380

B. In seven young men.

		lbs.	cu. in.	cu. in.	cu. in.	cu. in	min.	min.
R.L. W.†	24	112	1.66	0.81	0.90	1.09	34.	108
E. F. S.*	29	142	1.81	0.27	0.63	0.86	48.	120
V. Me.	28†	142‡	1.75	0.67	0.85	0.93	58.	135 (5¾ cc.)
C. E. F.*	26	150	1.49	.01	0.20	0.62	80.	140
B. J. A.*	31	154	1.68	.28	1.05	1.27	24.	90
C. C. C.*	26	160	2.10	.66	1.20	1.32	24.	105 (7 cc.)
L.O.Mo†	26	175	2.14	1.44	1.81	1.91	11.	56
Totals	190	1038	12.63	4.14 (67.9 cc.)	6.64 (108.9 cc.)	8.00 (131.2 cc.)	279.	754

C. Comparison of averages in two sexes.

Sex	Age	Wt.	Initial volume of bile	Total discharge of bile			Minimum time required to discharge	
				at 15' pc.	at 30'	at 45'	1 cu. in. (16.4 cc.)	All but 3 cc.
		lbs.	cc.	cc.	cc.	cc.	min.	min.
Average for 7 women	27—	137	24.3	14.1	17.1	19.3	23.	54
Average for 7 men	27+	148	29.5	9.7	15.5	18.7	40.	108

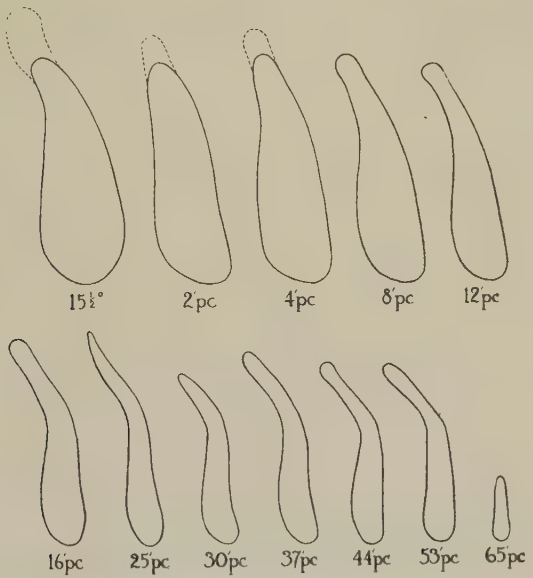
* For complete data, see earlier paper.[1]

† For effect of diet of milk or cream alone on these same individuals, see previous paper.[2]

‡ Estimated.

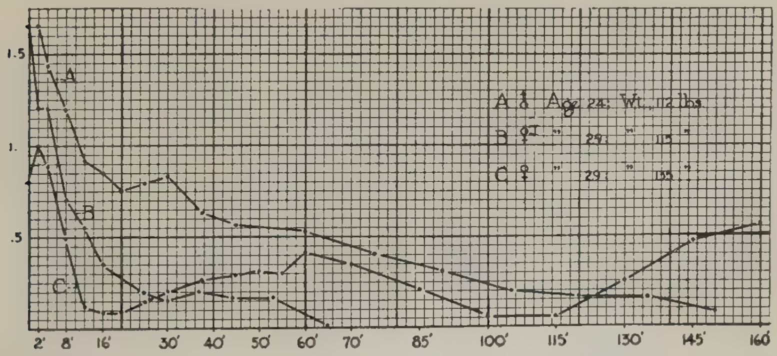
As regards individual comparisons, the most striking examples are shown in figure 4. Here are two healthy individuals of comparable age and weight, with gall bladders of the same shape and size at the beginning of experiment. That of the young woman (B) expels all but 3 of its 27 cc. of bile in the first half hour; that of

Fig. 3.



Tracings of cholecystograms showing typical contraction of gall bladder in a young woman (Dr. C. L. Birch). X 2/5. Volumes recorded in graph B, figure 4. 15½, 15½ hours after oral administration of tetraiodo-phenolphthalein; *2pc'*, 2 minutes after ingestion of egg-yolk and cream. *Dash line,* neck of gall bladder. Note change in shape following ingestion of food.

Fig. 4.



Graphs *A* and *B* (R. L. W. and C. L. B.) afford comparison of the contraction time of 2 gall bladders of the same size and shape, from individuals of the same weight but opposite sex. Graph *C*—Contraction curve of gall bladder in C. H. S. (Table I A), showing 2 successive emptyings within 1 hour and 40 minutes after a meal of egg-yolk and cream.

the young man (A) requires nearly four times as long (108 minutes) to discharge the same amount of bile. These graphs should also be compared with that of B. J. A. (Fig. 2; also Figs. 5 and 7 of a previous paper[1]), who required three times as long (90 minutes) starting with a gall bladder of the same shape and size as the one at B.

TABLE II.
*Volume of Distended Gall Bladder.**

A. Students X-rayed by the writer.

No. cases	Average age	Average wt.	Average vol.	Ratio of bladder bile to body weight.
		lbs.	cu. in.	
9 men	28—	149	2.72	1 cc.: 3 1/3 lbs.
9 women	26+	136	1.73	1 cc.: 4 3/4 lbs.

B. Dispensary patients.†

No. cases	Average age	Average wt.	Average vol.	Ratio of bladder bile to body weight.
5 men	40—	144	2.4	1 cc.: 3 3/5 lbs.
7 women	36—	119	1.45	1 cc.: 5 lbs.

* Volume of fundus, body and infundibulum of gall bladder during fasting, computed by methods described in earlier paper.[1]

† From cholecystograms obtained through the courtesy of Doctor Hartung.

In conclusion, one may say that no satisfactory explanation of these sex differences is at present available. Conceivably, the female gall bladder may possess a more powerful *tunica muscularis*. It seems more likely, however, that it is not different in structure, but is merely differently timed to meet the needs of digestion. The degree of contraction depends, for instance, upon the behavior of the *sphincter papillae*, the rate at which fatty foods leave the stomach, etc. Yet I am informed by competent physiologists that no sex differences of this sort have ever been observed. Perhaps the explanation will be forthcoming when we know more about the effect on the gall bladder of such conditions as acid stomach, chronic constipation and intestinal obstruction, a phase of the problem which is now under investigation.

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[1] Boyden, E. A., *Anat. Rec.*, 1926, xxxiii, 201-256.

[2] Boyden, E. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 157-162.

New York Meeting.

Cornell University Medical College, January 19, 1927.

3372

Is the Manoilov Reaction a Better Test for Metabolic Level Than for Sex?

OSCAR RIDDLE AND WARREN H. REINHART.

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In preliminary work with the Manoilov sex test we noted that a wrong or "reversed" reaction was rather frequently obtained when the test was applied to two blood samples from an adult pair of reproducing pigeons. These reversals then seemed to occur more frequently in those pairs which were *incubating eggs* at the time the test was made, and it was therefore decided to make a special study of the conditions attending these reversals. We designate as "reversals" those cases in which the comparison of the bloods from the two donor birds indicated the opposite of their actual sex. Conducting the study along these lines, in an animal in which many important details of the current reproductive states and processes are easily known, we have obtained results which we conclude are a contribution not only to this little understood reaction, but to the general problem of the nature of sexual difference.

When male and female mates are studied at a "resting stage" of the reproductive cycle (when no eggs are about to be laid, and when the pair is not incubating eggs) the Manoilov test gives a correct indication of the sex of the two donor birds in about 85 per cent of the tests (table 1). If, however, the blood be taken from the birds at or near the time eggs are ready to be released from the ovary, only 29 per cent of the tests give a correct answer. Also, while the birds are incubating eggs 2 to 12 days only 35 per cent of males and females are correctly placed by the test. Thus, under these two conditions, more males are diagnosed as females than as males, and *vice versa*. At the very end of incubation about 73 per cent of the cases are correctly placed by the test.

TABLE I.

Percentage of correct and reversed tests at various stages of the reproductive cycle.

Description	Resting stage		Ovulation period		2-12 days incubation		13-18 days incubation	
Ring doves								
Normal (♂ lighter than ♀)	85.0	17	27.8	5	40.0	8	81.8	9
Equal	——	—	11.1	2	——	—	9.1	1
Reversed (♂ darker than ♀)	15.0	3	61.1	11	60.0	12	9.1	1
Common pigeons								
Normal (♂ lighter than ♀)	84.6	11	30.8	4	28.6	4	50.0	2
Equal	——	—	7.7	1	7.1	1	25.0	1
Reversed (♂ darker than ♀)	15.4	2	61.5	8	64.3	9	25.0	1
Average (all pairs)								
Normal	84.8	28	29.0	9	35.3	12	73.3	11
Equal	——	—	9.7	3	3.0	1	13.3	2
Reversed	15.2	5	61.3	19	61.7	21	13.3	2

An important part of our method of study rests upon the utilization of a series of color standards (we used various dilutions of nicotine, to each of which the Manoilov reagents were added), such as would enable us to assign a definite value to the grade of color found (grades 1 to 14; 14 being colorless) in every blood test. The values thus obtained permit us to know whether it is the blood of the male or of the female, or of both, that undergoes change during incubation, egg-laying, etc. The figures found (table 2) show that for ring doves at the "resting" stage the average male stands at 10.5 in our scale, the female at 8.9. During "egg laying" and "incubation," however, the male figure drops to 9.2 and 9.0, while the female then becomes 9.7 and 8.4. At 13 to 18 days of incubation the values for male and female are lowest, 8.9 and 7.4, respectively. Less extensive data for common pigeons, a different zoological family, essentially confirm these findings in ring doves.

TABLE II.

Average values found for males and females in terms of a standard nicotine solution (N value).

Sex	Resting stage		Ovulation period		2-12 days incubation		13-18 days incubation	
	N value	No. tests	N value	No. tests	N value	No. tests	N value	No. tests
Ring doves								
Male	10.5	41	9.2	26	9.0	29	8.9	8
Female	8.9	37	9.7	30	8.4	28	7.4	10
Common pigeons								
Male	7.2	22	6.0	11	6.4	13	7.2	5
Female	6.4	16	7.4	12	7.4	14	5.8	3

The available facts suggest that the above values and periodic changes are best understood on the basis of known or probable metabolic levels, and periodic metabolic changes, in the sexes during the reproductive cycle. There is much evidence that among pigeons (as in at least most other organisms) the metabolic rate is higher in males than in females; and we may take our normally higher values (less coloration) for males, as an expression of this difference. Males become relatively inactive while incubating (the male pigeon nests during the daytime and is always inactive at night). This is therefore a period in which the current metabolic rate or level probably decreases in the male. The copulatory exhaustion of the male, at or before "ovulation" in the mate, would also probably lead to a decrease in his metabolic rate at that period. At both of these periods (ovulation; incubation) such a decrease, certainly a change toward the normal female level, is actually found.

The changes in the female may be explained as follows: Earlier studies by Riddle[1] have shown that when a female pigeon approaches the ovulation period the suprarenals temporarily enlarge; and Riddle and Honeywell[2] found evidence of increased adrenin secretion at this period as indicated by an increase of the blood sugar. Such increased adrenin secretion in the female should cause an increase in her metabolism at this period, and our figures for this period actually show an approach to normal male values. During incubation the female also, though she incubates chiefly at night, is probably less active and probably attains a lower metabolic level than during normal periods; the values found here fall below the normal. Our data thus suggest that the Manoilov "sex test" is really a better test of metabolic rate or level. According to this view it becomes a relatively good sex test also, in certain unmodified sexual periods, because metabolism and sex are intimately related in the way long urged by the senior author.

The amount of color remaining at the conclusion of the oxidation-reduction processes involved in the Manoilov test undoubtedly rests upon the amount of certain *substances*—probably reducing substances—in the substrate. Our hypothesis is that such substances tend to vary parallel with metabolic rate. Studies of a different nature with this reaction, to be described later, in part strongly and definitely support the above explanation; another part of those results are less easily interpreted.

A further fact is made clear by the data presented here. No sex-specific substance is involved in the Manoilov test, but the differential end-points of color rest plainly and purely upon a *quantitative* basis. We are anticipated in the publication of this conclusion by Alsterberg and Häkannson[3], whom we therefore confirm. It should be noted that this quantitative, not qualitative, character of the test conforms perfectly with our identification of the test as, primarily, one for metabolic levels. The metabolism of males and females is qualitatively essentially similar; it is normally quantitatively different in the sexes, but metabolism may suffer wide temporary fluctuations in either sex while the differentiated structures declaring the actual sexuality remain relatively fixed.

On the basis of the view developed here it is thought possible to explain the numbers, and the variable numbers, of failures of the test as these are recorded by all who have used the Manoilov reaction as a sex test. Investigators have frequently indicated the several physiological similarities (age, position of leaves on trees, etc.) that must exist in the male and female tissue compared. Such differences plainly involve, or at least suggest, metabolic differences.

The results indicate that the Manoilov sex test is a better test of metabolic level than of sex. From individual pairs of pigeons, on which repeated tests were made, the sex was usually correctly indicated by the test in ordinary life stages, but much more often incorrectly indicated at those particular reproductive stages in which metabolic change is known or probable.

The test rests purely upon a quantitative, not a sex-specific qualitative basis. In this we confirm Alsterberg and Häkannson. This fact, however, is necessary to and is in conformity with our association of the test with metabolic state or level.

The few or numerous failures of the test as a sex test are explainable in terms of the view developed here.

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[1] Riddle, O., *Am. J. Physiol.*, 1923, lxvi, 322.

[2] Riddle, O., and Honeywell, H. E., *Am. J. Physiol.*, 1923, lxvi, 240.

[3] Alsterberg, G., and Häkannson, A., *Biochem. Z.*, 1926, clxxvi, 251.

3373

Correlation of Toxicity of Normal Globulins with Increased Partition of Mid and End-Piece of Alexin.

EARL B. MC KINLEY.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

Bordet[1] has demonstrated that the intravenous injection of globulins from fresh, normal guinea pig serum into guinea pigs is followed by extreme shock and death of the animal, usually within 3 or 4 minutes. In 1925, together with Bordet, further study was made of this phenomenon.[2] In this study it was found that the toxicity of the globulin fraction of guinea pig serum for guinea pigs was destroyed when heated at 70° C. for 1/2 hour; that the toxicity of the globulin fraction remained intact when heated at 60° C. for the same length of time; that the leucocyte count of the animal was decreased to about 1/2 following injection; that the temperature of the animal increased 3 to 4 degrees and there was a delay in the coagulation time of the blood following injection, in some cases as long as 24 hours. At the same time it was shown that injections of globulins intraperitoneally or subcutaneously into guinea pigs were innocuous.

Later, in collaboration with Zunz[3], it was demonstrated that following the intravenous injection of globulins from guinea pig serum into guinea pigs there was, in some cases, a definite lowering of the surface tension of the plasma. This lowering of the surface tension it was thought might be due to the presence of hemoglobin in the plasma, and this, in turn, was accounted for by the alkalinity or acidity of the injected material. Zunz[4] has shown that the presence of hemoglobin notably lowers the surface tension of both water and plasma.

Our method of precipitating the globulins from guinea pig serum consists in diluting with 20 volumes of water and passing CO_2 gas through the mixture several times. After centrifugalization for 10 minutes the supernatant fluid is removed and the precipitate is dissolved in physiological salt solution to the volume of the original serum employed. The mixture is then made slightly alkaline with Na_2CO_3 to insure greater solubility. Such preparations are usually toxic for guinea pigs, in doses ranging from 1 1/2 cc. to 4 cc. when given intravenously, and usually produce shock within a few minutes, followed by death of the animal. It has been our experience that not all such preparations are toxic and this we have been unable to explain. However, upon the addition of lipoids to the 20 volumes of distilled water (lipoids from 1 cc. of syphilitic antigen after evaporation of alcohol for each 5 cc. of serum) it was found that such preparations were invariably toxic. In a few instances following the injection of such material we have found at autopsy changes in the lungs which closely resemble true anaphylactic shock.[3] This, however, is by no means the rule, as the phenomenon as first described by Bordet is quite different.

The role of lipoids and their effect upon the biological reactions of serum is little understood. Why should the addition of a small amount of lipoid to the distilled water before CO_2 precipitation always insure a toxic product, when without this addition various samples of globulins from sera are found to be non-toxic, although their preparation has been precisely the same in every instance? We might conceive of lipoid in this instance as an adsorption compound coating the protein molecule, followed by a more rapid and complete precipitation. The pH of our preparations are quite uniform, though the isoelectric point might conceivably be altered somewhat by our method. Powis[5] and Northrop[6] have observed that the critical potential of a suspension of sheep cells is raised by the presence of paraffin oil. Northrop regards this effect as closely analogous to the more general one of "protective colloids."

Since we are dealing here with the mid-piece of complement (end-piece is not toxic) it is suggested that the uniform toxicity of mid-

TABLE I.

Effect of addition of lipoid to distilled water before precipitation of mid-piece from normal guinea pig serum with CO_2 gas.

5% susp. of sheep cells	Salt sol.	1-200 sensitizer	Alexin 1-10	With lipoid		Without lipoid		Reading	
				Mid-piece	End-piece	Mid-piece	End-piece	30" 37° C.	12 hrs.
.05 cc.	1 cc.	.2 cc.	.1 cc.	—	—	—	—	CH	CH
"	"	.3 cc.	"	—	—	—	—	CH	CH
"	"	—	"	—	—	—	—	N	N
"	"	.2 cc.	—	.1 cc.	—	—	—	N	N
"	"	"	—	.2 cc.	—	—	—	N	N
"	"	"	—	—	.1 cc.	—	—	N	PH
"	"	"	—	—	.2 cc.	—	—	N	CH
"	"	"	—	—	—	.1 cc.	—	N	PH
"	"	"	—	—	—	.2 cc.	—	N	PH
"	"	"	—	—	—	—	.1 cc.	N	PH
"	"	"	—	—	—	—	.2 cc.	N	CH
"	"	"	—	.1 cc.	.1 cc.	—	—	PH	CH
"	"	"	—	.2 cc.	.2 cc.	—	—	CH	CH
"	"	"	—	—	—	.1 cc.	.1 cc.	PH	CH
"	"	"	—	—	—	.2 cc.	.2 cc.	CH	CH

piece, obtained when lipoids are added to the first distilled water, might be due to a more complete separation of mid-piece from end-piece than one ordinarily gets with the usual methods employed. This hypothesis has been tested in a hemolytic system to determine if such is the case. The following protocol will serve to illustrate the results obtained in a series of experiments:

TABLE II.

Effect of addition of Na_2CO_3 to final solution of mid-piece prepared with and without lipoids.

5% susp. of sheep cells	Salt sol.	1-200 sensitizer	Alexin	With addition of lipoid and Na_2CO_3		Without lipoid but with Na_2CO_3		Reading	
				Mid-piece	End-piece	Mid-piece	End-piece	30 min.	12 hrs.
.05 cc.	1 cc.	.2 cc	—	.1 cc.	—	—	—	N	N
"	"	"	—	.2 cc.	—	—	—	N	N
"	"	"	—	—	—	.1 cc.	—	N	N
"	"	"	—	—	—	.2 cc.	—	N	N
"	"	"	—	.1 cc.	.1 cc.	—	—	PH	CH
"	"	"	—	.2 cc.	.2 cc.	—	—	CH	CH
"	"	"	—	—	—	.1 cc.	.1 cc.	PH	CH
"	"	"	—	—	—	.2 cc.	.2 cc.	CH	CH

CH—Complete hemolysis. PH—Partial hemolysis. N—No hemolysis.

In Table I after 12 hours there was no hemolysis in the tubes which had received mid-piece precipitated from guinea pig serum, with addition of lipoids to the 20 volumes of distilled water before CO_2 gas precipitation. The tubes which had received mid-piece precipitated without addition of lipoids, showed partial hemolysis. End-piece in both cases after 12 hours produced complete hemolysis. This would seem to indicate that where lipoids are added to the distilled water used for diluting the serum before CO_2 precipitation there is a more complete separation of the mid-piece from end-piece. That is, there is little or no end-piece carried down with mid-piece, or at least a quantity below that which is necessary to act with mid-piece in producing hemolysis. Whether this is due to the rapidity of precipitation, or to a selective action of the lipoids for the globulin molecules as opposed to the albumin, we are not certain. In this experiment the final solution of mid-piece was on the acid side of neutral. Table II illustrates, at least in several experiments, of which this is a type, that when the pH of the mid-piece is slightly on the alkaline side, either with or without the addition of lipoids in their preparation, there is apparently complete separation, though these results are by no means regular. This again may simply indicate that there is not sufficient end-piece present to act with mid-piece in bringing about hemolysis and not that there is a complete absence of end-piece. It is, of course, apparent that these experiments are only rough titrations of hemolysis.

On the whole, it is indicated by these experiments that probably there is no greater separation of mid-piece from end-piece brought about by the presence of lipoid in the distilled water dilution of the serum than there is without lipoid. If there was greater separation we would have expected a difference in Table II between the two preparations. This did not occur. The uniform toxicity of globulins from normal guinea pig serum (prepared with lipoid) for guinea pigs is not to be explained upon this basis. It is suggested that the character of the precipitate may in some manner be modified and that the resulting solution-suspension of globulins is found in a different "colloidal state" which contributes to the toxicity of the preparation.

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[1] Bordet, J., *Compt. rend. Acad. sc.*, 1924, clxxix, 243.

[2] Bordet, J., and McKinley, E. B., *Compt. rend. Soc. biol.*, 1925, t. xcii, 762-764.

[3] McKinley, E. B., and Zunz, Edgard, *Compt. rend. Soc. biol.*, 1925, t. xciii, 459.

[4] Zunz, Edgard, *Bull. Soc. roy. Sc. med. et nat.*, Brussels, 1906, t. lviv, 187-203.

[5] Powis, F., *Z. physik. chem.*, 1914-15, lxxxix, 179, 186.

[6] Northrop, John H., *J. Gen. Physiol.*, 1924, vi, 603.

3374

Effect of High Voltage Cathode Rays on Rickets and on the Activation of Cholesterol.

ARTHUR KNUDSON AND W. D. COOLIDGE.*

From the Laboratory of Biological Chemistry, Albany Medical College, and Research Laboratory, General Electric Co., Schenectady, N. Y.

It has been shown repeatedly that ultraviolet radiation brings about healing of rickets and also that irradiation of cholesterol by ultraviolet light renders it antirachitic. Recently, one of the authors[1] has developed a means for producing high voltage cathode rays outside of the generating tube. It seemed interesting to study the effect of these rays on experimental rickets in rats, first when applied directly, and second when used for the activation of cholesterol.

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[*] With the assistance of H. E. Tanis, Jr., and F. S. Randles.

III. When cells are placed in the dye solution containing 0.05 M sodium chloride at pH 8.2, there is a decrease in the rate of penetration of dye, which is less than the decrease with the dye solution containing magnesium or calcium chloride at pH 7.9, though the dye solution contains the same proportion of borax to boric acid in both cases. This decrease may be interpreted in the same way as in section II.

IV. When cells are previously exposed to 0.05 M (*a*) sodium chloride, or (*b*) magnesium or calcium chloride, and then placed in the dye solution at pH 7.9, containing 0.05 M magnesium or calcium chloride, there is a decrease in the rate of penetration of dye as compared with the control,[5] but the extent of this decrease is slightly less than when cells are placed in the same dye solution without previous exposure to salt. Furthermore, the decrease in the rate of penetration is less in the case of cells previously exposed to sodium chloride solution and then placed in dye solution at pH 7.9, containing no salt other than buffer salts.

V. When cells are previously exposed to 0.05 M (*a*) sodium chloride, (*b*) magnesium chloride, (*c*) calcium chloride, and then placed in the dye solution containing 0.05 M sodium chloride at pH 8.2, the rate of penetration of dye is practically the same as that of the control,[5] with (*a*), and shows possibly a slight increase with (*b*), and a very slight decrease with (*c*).

These experiments indicate that the method of adding salts to the dye introduces variables (such as changes in the dye and in its effect on the protoplasm) which make the interpretation difficult. To study the effect of salts on the protoplasm it seems better, as previously suggested by the writer,[3] to expose the cells to the action of salts and then place them in dye made up with as little salt as possible.

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[1] All salts are dissolved in distilled water unless otherwise stated. Living cells are exposed to the salt solutions for 10 minutes, during which time no irreversible injury is brought about.

[2] In all cases, 0.00014 M dye solution is made up in M/150 borate buffer mixture (borax plus boric acid). The pH values are determined by means of the hydrogen electrode. Cells are placed in the dye solution for one-half minute.

[3] This result confirms the result given in the writer's previous papers, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 54, and *J. Gen. Physiol.*, 1926-27, x, 271.

[4] The concentration of dye in the vacuole is determined colorimetrically.

[5] Experiments where the cells are transferred directly from tap water to the dye solution containing no salt (except buffer salts) represent the control in all cases.

[6] The action of hydroxyl ions at such pH values on the dye has been found to increase the relative amount of the more readily diffusible form of dye, free

base, so that if they have an inhibiting effect, in presence of such chloride, their action on the dye must be different in presence of such salts.

[7] Addition of different concentrations of sodium chloride to the dye solution at pH 7.9 containing 0.05 M calcium chloride, diminishes progressively the extent of this decrease.

[8] The conflicting results obtained by various investigators seem to be due to the use of this method.

3385

On the Experimental Transmission of Arsenic to the Aqueous Humor.

A. C. KRAUSE, A. M. YUDKIN AND D. G. MORTON.
(Introduced by S. C. Harvey.)

From the Ophthalmological Section of the Surgical Department, Yale University School of Medicine.

This investigation is a study of the arsenic content of the aqueous humor after the intravenous injection of neo-arsphenamine in dogs.

Eleven dogs were used. Fifty milligrams of neo-arsphenamine per kilogram of body weight was injected into the jugular vein of each dog. The eyes were anesthetized with cocaine. As much aqueous humor as possible was removed from each eye with a fine bore needle at varying intervals of time after injection. The fluids of the two eyes were pooled in every case. After digestion[1] with acid, a modified Gutzeit method[2] was used in the determination of the amount of arsenic in the samples.

The details and results are shown in the accompanying table.

TABLE I.

Dog No.	Injection of 50 mg. of neo-arsphenamine per kilogram.							
	Before inject.	After Injection						
	0.5 hr.	1 hr.	2 hr.	4 hr.	8 hr.	24 hr.	48 hr.	96 hr.
1	0	8	20	15		2	0.6	
2	0	7	16	13		2	1	
3		T	24	15		2		
4		T	21	16		1	1	
5			T	9		1.5	0.8	
6				T	2.5	1	0	
7						1	2	0.5
8							1	0
9		T		5		1.5	T	
10		T			2.	T	T	
11		T				T	T	

T represents trace. Results in micromilligrams or arsenic trioxide.

The normal aqueous humor contains no arsenic. Only a trace of arsenic penetrates in the anterior chamber of the eye during the first few hours after intravenous injection of large doses of neo-arsphenamine, and only the smallest measurable amount after 24 hours, representing such a slight increase over the first few hours that it seems of no significance. Paracentesis definitely increases the permeability of the eye to arsenic, even though it be done a half hour *before* injection of the drug. It was found that the amount of arsenic in the aqueous humor of subsequent tappings was less than that in the previous determination. The bulk of arsenic which accumulates after paracentesis apparently disappears within a few hours.

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[1] Robertson, G. R., *J. R. Am. Chem. Soc.*, 1921, xliv, 182.

[2] Allen, W. S., and Palmer, R. M., General Chemical Company.

3386

Determination of Albumin and Globulin in Urine.

ALMA HILLER. (Introduced by Donald D. van Slyke.)

From the Hospital of the Rockefeller Institute for Medical Research, New York.

Albumin and globulin are separated by precipitating the latter with sodium sulfate, as in Howe's[1] technique for plasma protein separation. The separated proteins are determined by the colorimetric method of Autenrieth,[2] which can be made practical for general colorimetry by introducing pure biuret as a standard. One mg. of biuret gives a color equal to that of 0.924 mg. of urinary proteins treated with alkali and copper sulfate, as described by Autenrieth.[2]

The standard solution is made by dissolving 0.4 gm. of biuret in water and diluting to 150 cc. Five cc., containing 13.33 mg. of biuret, is colorimetrically equivalent to 12.3 mg. of urinary proteins.

For total protein, enough urine to contain 8 to 20 mg. is precipitated with an equal volume of 10 per cent trichloroacetic acid. The precipitate is redissolved in 3 per cent NaOH, treated at 10 cc. volume with 0.25 cc. of 20 per cent $CuSO_4 \cdot 5H_2O$, and compared with 1 cc. of the biuret standard similarly treated.

Globulins are precipitated by treating the urine at 38° with an equal volume of 44 per cent Na_2SO_4. In the filtrate, the albumin is precipitated with trichloroacetic acid, and determined as described for total proteins. Globulins are calculated as (total protein)—(albumin).

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[1] Howe, P. E., *J. Biol. Chem.*, 1921, xlix, 93, 109.

[2] Autenrieth, W., and Mink, F., *Münch. med. Woch.*, 1915, lxii, 1417; Autenrieth, W., *Münch. med. Woch.*, 1917, lxiv, 241.

3387

The Cause of Temporary Ventricular Alternation Following a Long Diastolic Pause.*

CARL J. WIGGERS.

From the Department of Physiology, Western Reserve University School of Medicine.

A condition of temporary alternation, such as can be induced in dogs' hearts which are normal, according to every criterion we are able to apply, was studied by apparatus recording pressure and volume curves by optical projection.

It was found that temporary alternation induced by the application of premature stimuli to a ventricle or an auricle, by temporary vagus inhibition or by a temporary a-v block, invariably involves both ventricles but does not affect the auricles. Neither the permanence nor duration of the alternation is affected by the volume of venous return or the height of the arterial pressure. Alternation appears to be conditioned entirely by the rate of beat, and cannot be induced in the normal dog's heart when beating at rates below a critical level of about 140 per minute.

The alternating beats resemble those of a more permanent nature induced in other ways. In both cases they differ from the normal, not only in their amplitude of contraction and volume of systolic discharge, but also in the contour of contraction and relaxation and in the duration of their phases. Thus, in the smaller beats the pressure rises more gradually, the isometric contraction phase is prolonged, while systolic ejection and total systole are abbreviated. The isometric relaxation also occurs more slowly so that the interval between the end of systole and the beginning of ventricular inflow is prolonged.

The alternate beats begin with different diastolic volumes and as a rule also with corresponding changes in initial tension. Thus, just before the smaller contraction begins the diastolic size is less and the initial tension is lower. The reverse obtains in the case of the larger beat.

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* A detailed report of this research will appear in the Warthin Anniversary Volume, George Wahr, Ann Arbor, 1927.

A critical study of the possible causes of such variations in filling leads to the conclusion that this is essentially due to the alternating extent of ventricular emptying. Thus, the residual volume retained after a small beat added to the natural inflow results in a larger diastolic volume and higher initial tension, in spite of the slight impediment offered to inflow. Changes in cycle length are obviously not concerned. Alternate changes in filling pressure or concordant variations in amplitude of auricular contraction do not occur. Nor do changes in ventricular relaxation play any rôle. Isometric relaxation, it is true proceeds more slowly after the smaller beats but this is so nicely neutralized by an earlier onset of relaxation that the duration of inflow changes not at all or is affected very slightly. Furthermore, the rate of inflow is slightly impeded after the smaller and not after the larger beat.

Recent observations have shown that such alternate changes in diastolic size and initial tension inaugurated by the longer pause can cause the amplitude, contour and duration of ventricular contractions to change in the same directions as in this form of alternation. It is, therefore, possible to attribute all the effects shown in such temporary alternation to changes in filling. More convincing evidence than we have at present is necessary before we can assume that any specific alteration in the function of ventricular muscle is necessary to produce this type of alternation.

Attention is directed to the fact that this interpretation of the alternation phenomenon may not be applied without further study to those clinical forms which arise under different circumstances.

3388

Action of the Sympathetic Upon the Excitatory Process in the Dog's Heart.

E. COWLES ANDRUS and LOUIS E. MARTIN.
(Introduced by Edward P. Carter.)

From the Cardiographic Laboratory of the Johns Hopkins Hospital and University.

It was the purpose of the present study to investigate the effect of the sympathetic nerves upon the development and propagation of the excitatory process in the dog's heart. After the vagus had been functionally excluded by atropin, the sympathetic endings were stimulated by means of adrenalin or paralyzed with ergotamine.

The intravenous injection of adrenalin, 0.25 to 0.50 cc. of 1/50,000 solution, brought about within one-half to one minute a conspicuous acceleration of the rate of beat, a slight shortening of the P-R interval, and an increase in the rate of transmission in the auricle of 3 to 400 mm. per sec. Ergotamine (0.25 to 1.0 mg.), on the other hand, caused consistently a slowing in rate, a lengthening of the P-R interval, and a delay in intra-auricular conduction. This effect, however, came on much more slowly than that produced by adrenalin, and lasted for from one to two hours.

SCIENTIFIC PROCEEDINGS.

Minnesota Branch.

University of Minnesota, Minneapolis, January 26, 1927.

3389

The Determination of Iodine in Natural Waters.

J. F. McCLENDON.

From the Laboratory of Physiological Chemistry of the University of Minnesota.

A large dishpan is filled with the water, 1 gram of sodium hydroxide is added and evaporated over a stove or gas burner. As the water evaporates, more is added until 100 litres have been added and evaporated to about 1 litre. After the addition of 2 drops of 60 per cent NaOH solution, it is filtered into a porcelain evaporating dish, evaporated to dryness, and heated to burn the organic matter, either over a free flame or in a furnace.* The temperature should not rise enough to make the dish luminous. It is cooled, 25 cc. of absolute alcohol added, and the residue rubbed with a pestle. The alcohol is decanted through a filter and 10 cc. more added. The process is repeated until 100 cc. have been added. The filtrate is evaporated in a porcelain dish after the addition of 1 drop of 60 per cent NaOH solution, and enough water to keep it in solution. When the volume has reached a convenient size, it is transferred to a platinum or nickel dish or crucible, and evaporated to dryness. The residue is dissolved in 2 cc. of distilled water, transferred to a small beaker, and after the addition of two drops of 0.1 N sulfuric acid, it is neutralized with phosphoric acid, using an indicator paper made by drying an alcoholic solution of brom-phenol-blue or methyl orange on ash-free filter paper. The solution is boiled to remove the CO_2 and SO_2, and cooled. The volume is made up to 10 cc. in transferring to a 12 cc. separatory funnel having a 10 cc. graduation mark. One cc. of purified carbon tetrachloride, and 1 or 2 mg. of sodium nitrite are added, and the separatory shaken 200 times.† Eighty-five ninety-fifths of the iodine passes into the carbon tetrachloride, and colors it pink or violet, but it is cloudy with water droplets. It is run into a 1 cc. glass stoppered bottle, centrifuged to remove the water and a colorimetric match made. Ten mg. of iodine are dissolved in 100 cc. of purified carbon tetrachloride, and into 10 cc. glass stoppered bottles are placed 1 to 10 cc., respectively, of this solution of iodine, and the volume made up to 10 cc. in each with purified carbon tetrachloride. Then 1 cc. glass stoppered bottles of the same size and shape as that used to centrifuge the unknown are filled with these different dilutions of the iodine solution. They contain 0.001 to 0.1 mg. of iodine per cc. The unknown is compared with these known solutions colorimetrically in order to determine the quantity of iodine extracted, but if there is more than 0.1 mg., the unknown solution may be diluted quantitatively to bring it within the range of the known solutions. Since only 85/95 of the total iodine were extracted in the separatory funnel, it would be necessary to multiply the result by 1.115 in order to obtain the total iodine of the sample, but this is usually superfluous with the above bottles. If a more accurate determination is desired, a micro-colorimeter made by the Bausch and Lomb Optical Company should be used. In this case only one standard (containing 0.1 mg. of iodine per cc.) is used. About 2 per cent of the iodine is lost by filtering the sample of water (that is being evaporated) at the time it reaches the volume of 1 litre, but a great deal of time is saved and the procedure is justified. Although the colorimetric determination may be made within 1 per cent by the use of the micro-colorimeter, taking the average of 5 or more readings, the absorption coefficient is not known so accurately. It is difficult to reach an absolute equilibrium with a separatory funnel. The method of repeated extractions in a separatory funnel may be used, each extraction estimated separately and the total added together. If it is desired to titrate the iodine rather than to use the colorimetric method, run 10 cc. of distilled water into a separatory funnel, add one drop of 0.1 normal sulfurous acid and the carbon tetrachloride containing the iodine, and shake 200 times or until all of the iodine is reduced to iodide and passes into the water, run off the carbon tetrachloride and transfer the water to a beaker, make volume 100 cc., acidify with 1 cc. of 50 per cent phosphoric acid, oxidize with chlorine or bromine water. Boil off the bromine or chlorine, transferring to a dry beaker to prevent bumping, and reducing the volume to 50 cc., add 2 cc. of KI and some starch solution and titrate with 0.001 N thiosulphate with a micro biuret, and divide the result by 6. This is a complete report.

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* Danger of overheating may be avoided by burning in a combustion tube with one end bent down into an NaOH solution to absorb iodine that may escape. The arrangement is shown in *J. Biol. Chem.*, 1924, lx, 293, except that a side-neck test tube and suction allow the cold end of the combustion tube to be left open.

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† Technical carbon tetrachloride may be sufficiently purified by coloring it with bromide or chlorine, allowing to stand in a glass bottle in sunlight for a week, washing out the chlorine or bromide with NaOH solution, water and then with distilled water, syphoning off the water, filtering through paper flooded with CCl_4 to remove water droplets, and finally through a layer of plaster of Paris and distilling, rejecting the cloudy first portion of the distillate. It may be tested by making up a standard solution of iodine, letting it set for a week and comparing it with a fresh solution as mentioned above. If the color fades, the carbon tetrachloride still contains a reducing substance. This may sometimes be removed by shaking it with a dilute solution of nitrous acid made by adding a little sodium nitrate to very dilute hydrochloric acid or adding a few drops of nitrosyl sulphuric acid to a large volume of distilled water. Nitrosyl sulphuric acid may be made by adding starch to nitric acid in a retort and distilling over the mixture into concentrated sulphuric acid.

3390

The Prenatal Growth of the Human Pancreas.

RICHARD E. SCAMMON.

From the Department of Anatomy, University of Minnesota.

The ponderal growth of the pancreas, with respect to body-weight, has been studied from 319 observations on human fetuses, varying from 26 to 4380 gm. in total body-weight. When the weight of the fetal pancreas is plotted against the weight of the body as a whole, it follows the course of a straight line which may be approximated by the expression:

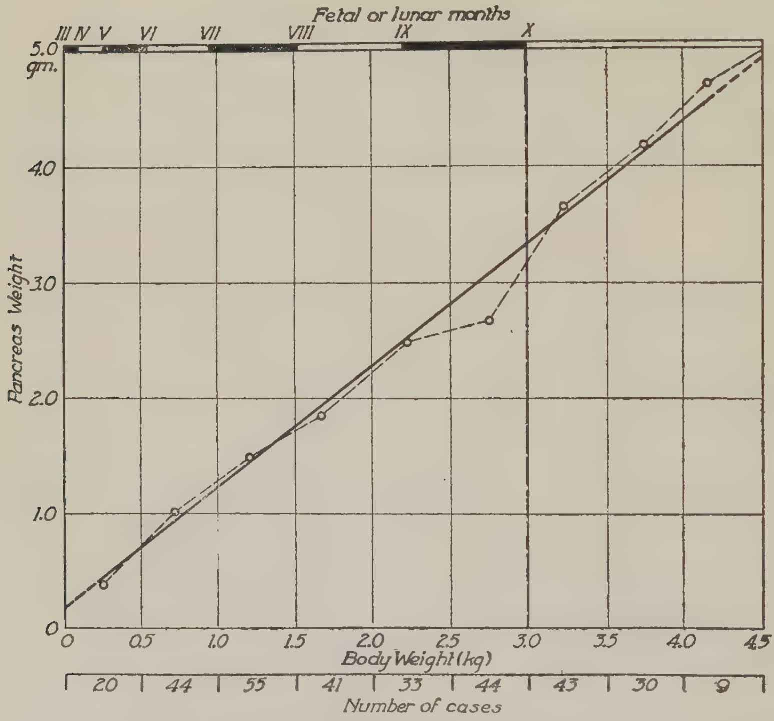
$$PW = 0.0010335\ BW + 0.19 \quad (1)$$

where "PW" is the pancreas weight in grams and "BW" is the total weight of the body in grams. This formula was computed from the means of 500 gm. ranges of body-weight, from 0 to 4500 gm. inclusive, by the method of means (weighting by the square root of the number of cases in each interval). The calculated values thus obtained show a mean, weighted, absolute, deviation of 0.123 gm. and a mean, weighted, relative deviation of 6.07 per cent from the observed averages. A similar relationship to total body-weight is characteristic of the weights of many of the other organs and parts of the body.

The relation of pancreas-weight to body-length, as determined from 325 observations, is shown in Figure 2. As in the case of the weight of the body as a whole and many of its parts, the weight of the pancreas may be approximated by a small fraction of the body-length raised to a power approaching a cube. The empirical formula, determined from the averages of pancreas-weight for 5 cm. intervals of body-length from 15 to 55 cm. inclusive (weighting by the square root of the number of cases in each interval) is:

$$\text{PW (gm.)} = 0.00001150 \text{ BL (cm.)}^{3.2035} \quad (2)$$

FIG. 1.

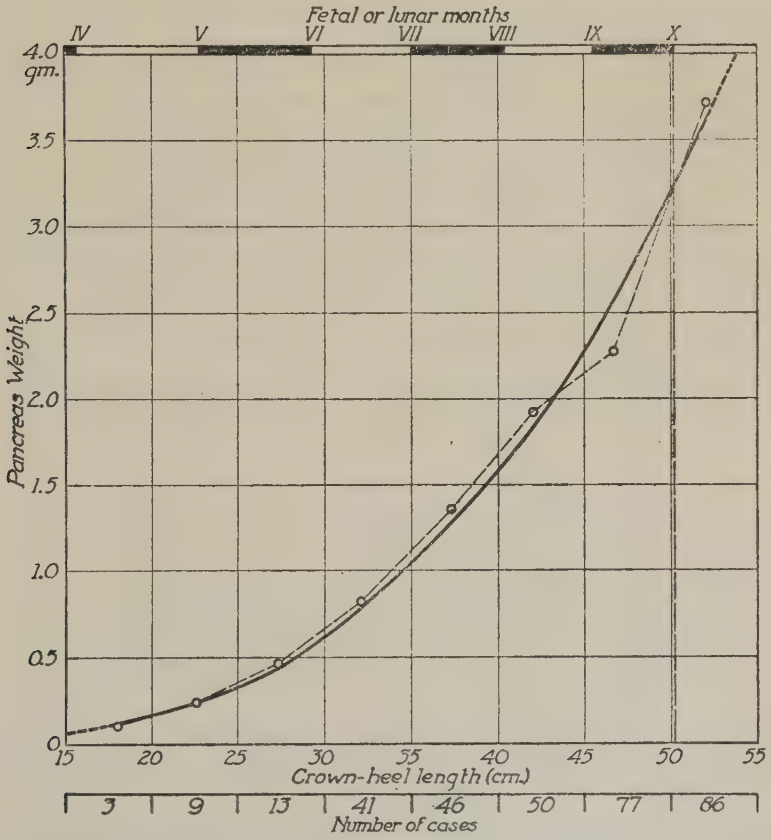


A graph illustrating the ponderal growth of the pancreas with respect to total body-weight in the fetal period. The body-weight (in kg.) is indicated on the base line of the graph. The estimated age (as computed from the "exact" formula of Scammon and Calkins) is represented by the panels along the upper margin of the figure. The observed, mean pancreas-weights for 500 gm. intervals of total body-weight are indicated by circles connected by light broken lines. The graphical expression of the empirical formula for the relation of pancreas-weight to body-weight is represented by the heavy solid line. The number of observations in each 500 gm. interval of body-weight is indicated in the lower panel of the graph. The vertical broken line in the field represents the computed birth value of body-weight.

where "PW" is the pancreas weight and "BL" is the total or crown-heel body-length. The mean, weighted, absolute deviation of the observed from the corresponding calculated means as determined by this formula is 0.121 gm., and the corresponding relative deviation is 6.710 per cent.

Applying the formula of Scammon and Calkins[1] for the relation of age to body-weight, the computed natal weight of the pancreas

FIG. 2.



A graph illustrating the ponderal growth of the pancreas with respect to crown-heel body-length. The body length (in cm.) is indicated on the base line of the graph. The estimated (as computed from the empirical formula of Scammon and Calkins) is represented by the panels along the upper margin of the figure. The observed mean pancreas weights for 5 cm. intervals of total body-length are indicated by circles connected by light broken lines. The graphical expression of the empirical formula for the relation of pancreas-weight to body-length is represented in heavy solid line. The number of observations in each 5 cm. interval of body-length is indicated in the lower panel of the graph. The vertical line in the field indicates the computed birth value of body-length.

is 3.29 gm. Applying the age-length formula of the same authors[2] the natal weight of the pancreas is 3.23 gm. A collation of natal pancreas weights from various sources (465 cases) gives a mean value of 3.54 gm., which is distinctly higher than the computed ones. As a rule the observed, average, natal weights of organs, as usually recorded, are lower than the calculated ones since the former often contain a number of premature cases. The reverse condition in this instance may be due to the exclusion of all known cases of congenital lues (which often increases the pancreas weight) from the present series of observations.

These figures indicate; (a) that the ponderal growth of the pancreas in the fetal period is quite comparable to that of the body as a whole, and that of most of its major parts and organs; and (b), that although the growth in absolute weight of the pancreas is proportional to the growth in body-weight in the fetal period, the *relative* weight of the organ, with respect to body-weight, undergoes a reduction during this period from 0.3 per cent at four fetal months to 0.1 per cent at birth. This is a complete report.

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[1] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 355, (formula 1).

[2] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 157, (formula 5).

3391

The Growth of the Arterial System in the Human Fetus.

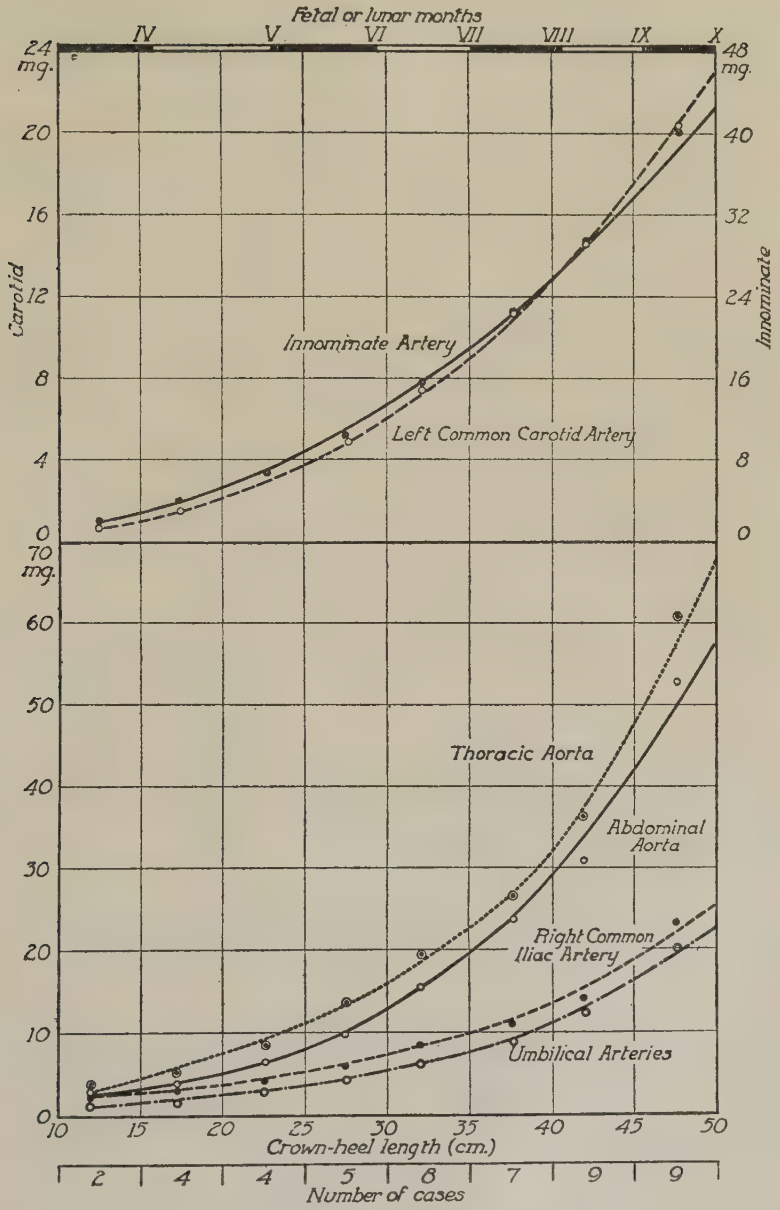
CHARLES H. WATKINS. (Introduced by R. E. Scammon.)
From the Department of Anatomy, University of Minnesota.

A quantitative study has been made of the growth of a portion of the arterial system in 48 human fetuses ranging from 10.7 to 49.8 cm. in total or crown-heel length.

A segment of standard length was cut from each vessel with an apparatus consisting of two steel blades firmly fastened in parallel to a machined brass block exactly 5 millimeters apart. Rings were cut from each of the following arteries: thoracic aorta, abdominal aorta, innominate, left common carotid, left subclavian, right and left umbilical and the right and left common iliac.

When the weights of the rings are plotted against crown-heel length in centimeters, the resulting curves are of two general classes. The values of the rings of the vessels which supply the body only,

FIG. 1.



A graph illustrating the growth in weight of rings of various arteries in prenatal life. The ring weights (in mg.) are plotted against the total body-length (in cm.). The observed means for the 5 cm. intervals of body-length are represented by dots or circles, and the lines drawn through them represent the calculated expressions given in this paper. The total body-length is given on the base line of the graph. The panels along the lower margin represent the number of cases in each 5 cm. interval of body-length. The panels along the upper margin represent the age in fetal or lunar months. The latter are calculated by the empirical formula of Scammon and Calkins[1] for the relation of fetal age to body-length.

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the innominate, left common carotid and left subclavian arteries, fall in the first class, in which the relation to body-length may be expressed by the general formula:

$$Y = aX^b$$

where "Y" = the weight of the segment in question, "X" = the total body-length and "a" and "b" are empirically determined constants, the first in the form of a decimal fraction and the second a power between a square and a cube. The graphs of these relationships are shown in the following figure. The empirical formulae for these weights, as determined from the 5 cm. interval averages of crown-heel length by the method of means, weighting by the square root of the number of cases, are as follows:

TABLE I.

Artery	Empirical Formula	Mean weighted absolute deviation	Mean weighted relative deviation
		(mg.)	(per cent)
Innominate	$W(0.1\ mg.) = 0.74121L(cm.)^{2.21827}$	0.72	2.11
Left Common Carotid	$W(0.1\ mg.) = 0.091892L(cm.)^{2.59054}$	0.13	1.37
Left Subclavian	$W(0.1\ mg.) = 0.050606L(cm.)^{2.74465}$	0.23	1.47

The curve of the left subclavian artery is not included in the figure for its values are so similar to those of the left common carotid artery that they could not be clearly represented upon the combined graph.

The weights of the rings of the vessels which supply both the body and placenta, the thoracic and abdominal aortae, the right and left umbilical, and the right and left common iliac arteries, form a second type in which the relation to body-length may be expressed by the general formula:

$$Y = a + bX + cX^2 + dX^3$$

where "Y" = the weight of the segment in question, "X" = the total body-length and "a", "b", "c" and "d" are empirically determined constants. The graph of these relationships are shown in the lower section of the following figure. The empirical formulae for these values, as determined from the 5 cm. interval averages of crown-heel length by the method of means, weighting by the square root of the number of cases are:

TABLE II.

Artery	Empirical Formulae	Mean weighted absolute deviation	Mean weighted relative deviation
		(mg.)	(per cent)
Thoracic Aorta	$W(0.1\text{ mg.}) = -148.2659 + 22.28314L(\text{cm.}) - 0.83921L^2 + 0.01456L^3$	1.40	4.32
Abdominal Aorta	$W(0.1\text{ mg.}) = 5.19394 + 2.53099L(\text{cm.}) - 0.14442L^2 + 0.00648L^3$	1.09	5.93
Mean of the Right and Left Umbilical	$W(0.1\text{ mg.}) = -0.84473 + 2.69173L(\text{cm.}) - 0.10109L^2 + 0.00299L^3$	0.46	2.43
Right Common Iliac	$W(0.1\text{ mg.}) = -31.08656 + 5.08158L(\text{cm.}) - 0.19437L^2 + 0.00392L^3$	0.42	1.97
Left Common Iliac	$W(0.1\text{ mg.}) = 9.62014 + 2.53324L(\text{cm.}) - 0.09946L^2 + 0.00275L^3$	0.67	4.79

The curve of the left common iliac artery is not included in the figure for its values are so similar to those of the right common iliac artery that they could not be clearly represented upon the combined graph.

The growth curves of the vessels which supply the body only show the same type of increment which is characteristic of the growth of most of the organs and parts of the body. These vessels have the short period of slow growth in early fetal life, followed by a more rapid increase during the rest of the fetal period.

The curves of the vessels which supply *both* the body and placenta are characterized by a relatively long period of slow growth followed by a short period of more rapid growth during the latter part of prenatal life. This is a preliminary report.

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[1] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1924, xx, 353.

3392

Observations on the Mechanism of Circulatory Failure in Diphtheria.

M. H. NATHANSON. (Introduced by George Fahr.)

From the Department of Medicine, University of Minnesota.

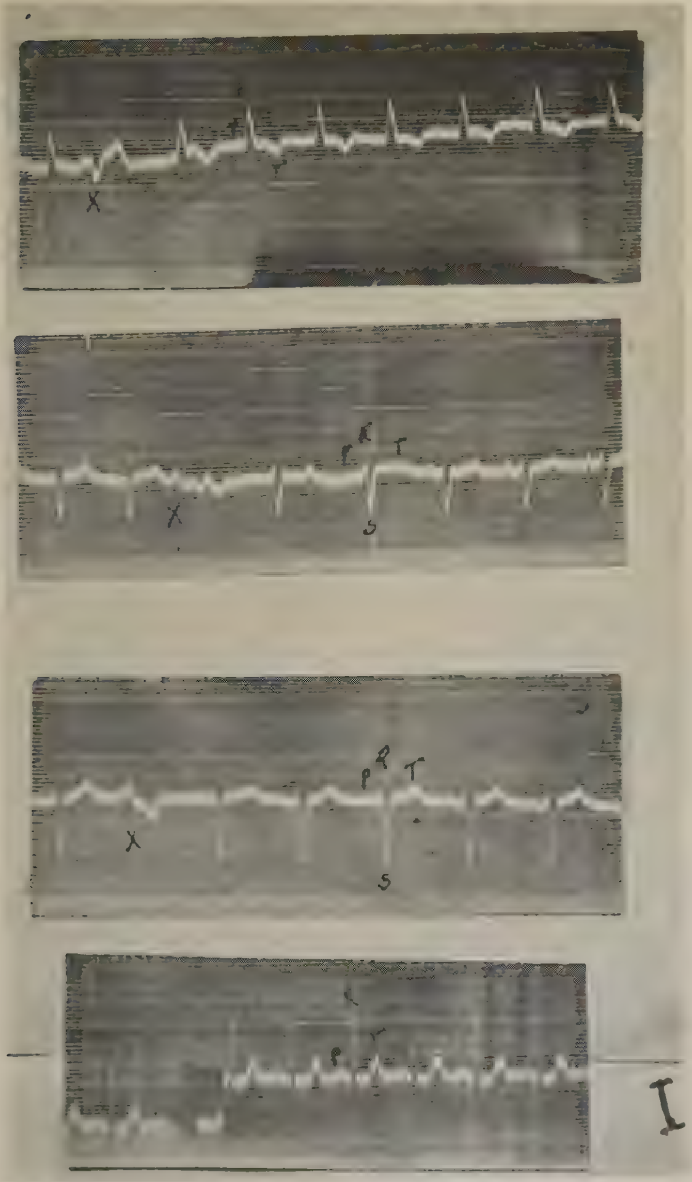
This report consists of study by means of the string galvanometer of the effects of diphtheria toxin upon the heart. It has long been known that a circulatory collapse may occur in diphtheria, which frequently results in death. There is still considerable uncertainty, however, as to whether it is the heart or the vasomotor apparatus which is primarily effected.

In the present study electrocardiographic observations were carried out (1) on a series of diphtheria patients (2) on animals injected with diphtheria toxin.

Previous observers[1, 2, 3, 4] have noted electrocardiographic changes indicating myocardial injury during the acute stage of diphtheria. The present investigation was made during the convalescent or late stage of the disease, since circulatory failure may occur during this period also.

Readings were taken of 15 diphtheria patients during the period of convalescence, beginning usually in the 3rd or 4th week of their illness. In all cases, the local throat condition had entirely cleared

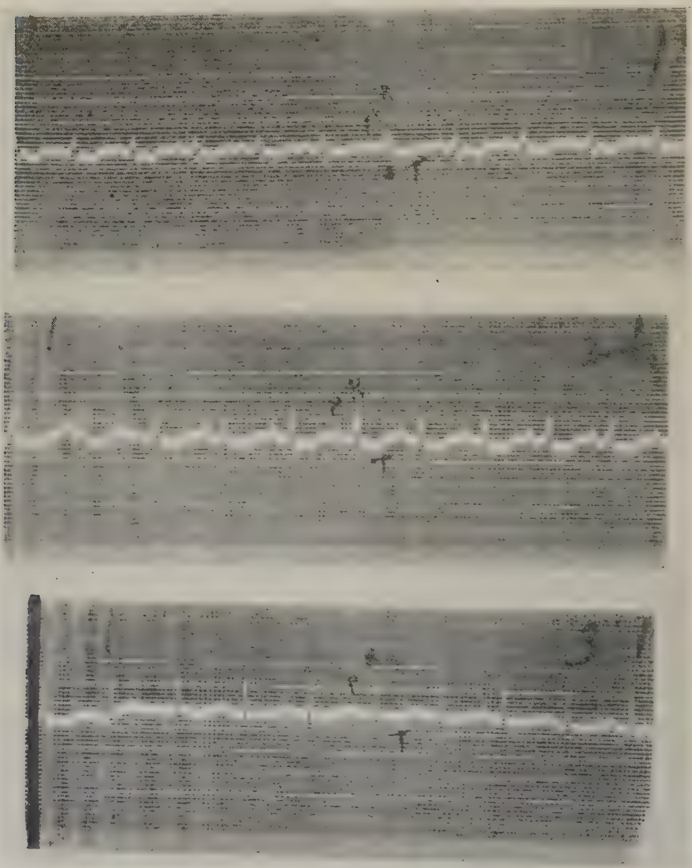
Fig. 1.



Electrocardiogram of a patient with multiple neuritis in the fourth week of convalescence. Inversion of T in lead one and ventricular extrasystoles. The lowest record shows lead 1, 3 months later with normal T.

up, and the cultures for the diphtheria bacillus were negative. The general symptoms of toxemia had disappeared, and practically the only abnormality was a heart which increased in rate very easily. Of the 15 cases, 8 had suffered a severe type of infection and of these, 7 showed significant abnormalities in the electrocardiogram. A summary of the readings shows the following: There was an absence of high-grade irregularities, occasional ventricular extrasystoles being the only arrhythmia noted in two cases. There was no evidence of prolonged auriculoventricular or intraventricular conduction. The longest P-R interval was .18 second and the

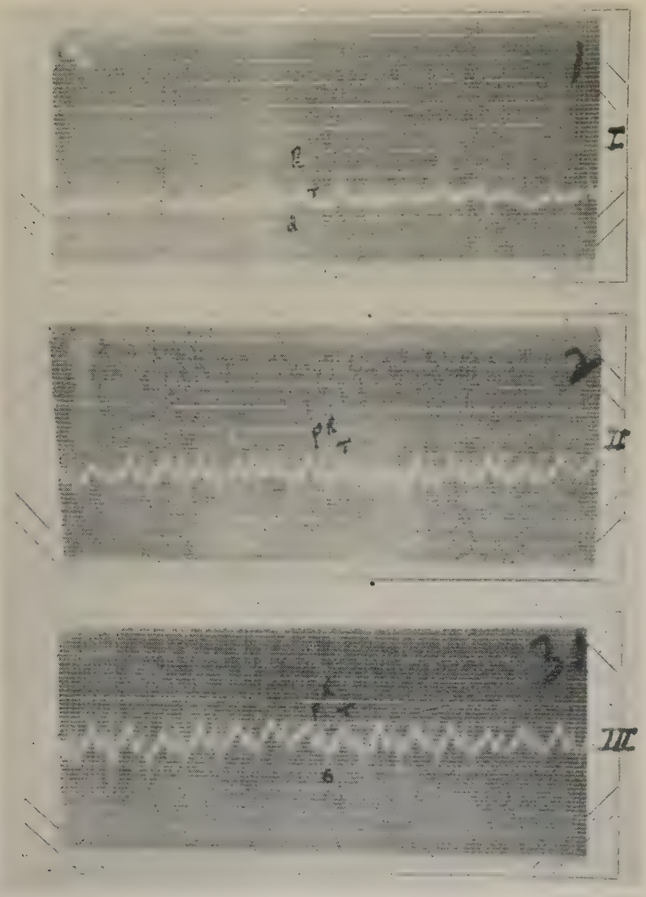
FIG. 2.



Inversion of T in all leads in the 4th week. The patient apparently in good condition, sat up to reach for a pitcher of water, 7 days after the record was taken, and died within a few minutes.

Q. R. S. complex was not altered in any case. The chief significant change was in the T wave. A definite inversion of the T was present in all. In one instance, the T was inverted in lead one, in another, in leads 1 and 2. In 3 cases, the change occurred in leads 2 and 3 and in 2, in all leads. In addition, 4 electrocardiograms showed a right preponderance, and 2 a left preponderance. In the 2 cases showing inverted T in all leads, sudden death occurred within a week after the readings were taken. The remaining 5 have recovered and with recovery the T wave has become upright. The change was a gradual one, the T first becoming less negative, then isoelectric and later positive. The earliest evidence of return to

Fig. 3.



Cat 3, before diphtheria toxin.

normal was noted 6 weeks after the onset of the disease. The return of the T wave to normal in the cases that recovered is of interest. It is still believed by some that diphtheria is a cause of cardiac failure in later life. The above observations strongly suggest that the effect is entirely a transient one, and that there is a restoration of normal myocardial function.

Rohmer,[1] in the course of an investigation upon the problem of whether diphtheria toxin possessed any special affinity for the conducting tissue of the heart, injected diphtheria toxin into 4 rabbits. In a brief paragraph, he merely states that he found no evidence of delayed conduction. He makes no note of any other changes. This seems to be the only previous report on the use of the string galvonometer in the study of experimental diphtheria.

In the present study, cats were used, and diphtheria toxin* injected subcutaneously. The dose of toxin varied from .03 cc. to .05 cc. diluted to 2.5 cc. in sterile saline. The records were taken with the animal under light ether anesthesia. After a control record was taken and the toxin injected, subsequent readings were made at 48 hours and at varying intervals following this, if the animals remained alive. All the animals exhibited definite evidence of intoxication. The effect was usually noted on the second day following the injection. The animals became apathetic, moved about very little and refused their food. A small amount of anesthetic was necessary in taking the subsequent readings as they offered but slight resistance. A very frequent observation was a weakness and stiffness of the hind legs which was almost a constant finding. This usually occurred on the 2nd or 3rd day.

Of 7 satisfactory experiments, electrocardiographic changes were observed in 6 after the injection of the toxin. These may be summarized as follows: No definite arrhythmias occurred. Definite changes in the P-R interval and Q. R. S. complex were present in but one case. In this animal, 6 hours before death, the record showed a P-R interval of .12 second, where it had previously been .06 second. At the same time the Q. R. S. had markedly increased in amplitude and had widened from .02 to .06 second. The most constant and striking alterations were in the T wave. There was a definite flattening and inversion of the T in one or more leads in all cases, as early as 48 hours following the injection of the toxin. The change was present in lead 1 alone, in 2 cats, and in leads 1 and 2 in one animal. In the remaining 3, the inversion was present in leads 2 and 3. In 2 cats in whom it was possible to follow for 7 and 10 days, a tendency for return of the T to normal appeared between the 3rd and 6th day in one instance; and between the 5th and 8th day in the other. In addition, in 2 animals, there was a definite change in ventricular preponderance, both within 48 hours, following the injection of toxin. In both cases a definite left ventricular preponderance developed. Similar rapid changes in preponderance are reported by McCulloch[2] and Marvin[4] in their clinical cases. In one case, a definite flaccid paralysis of the hind extremities was present on the 10th day, when the electrocardiographic changes were returning to normal. This is of interest since clinically, diphtheretic paral-

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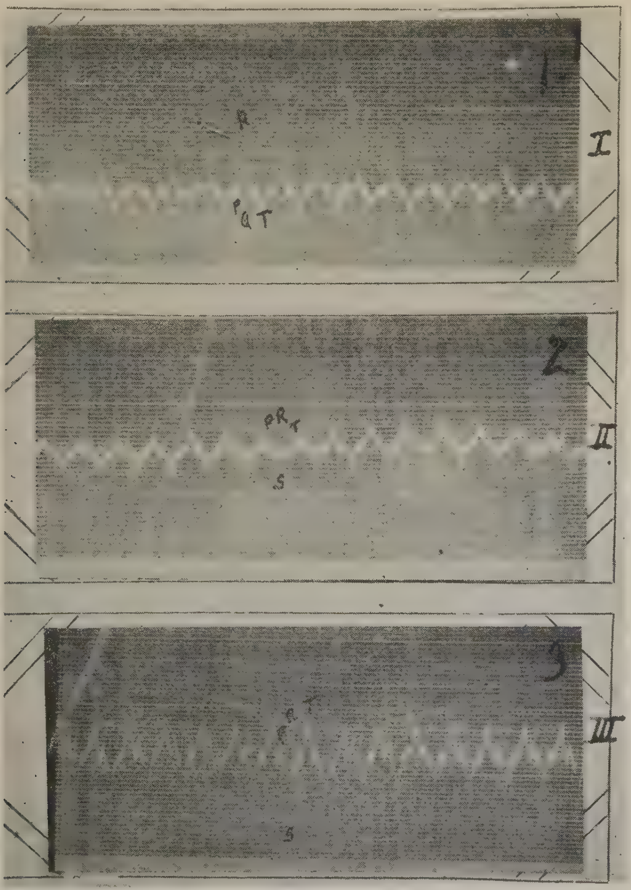
* The toxin was supplied by Dr. W. P. Larson of the Department of Bacteriology. ML dose equals 0.4 cc.

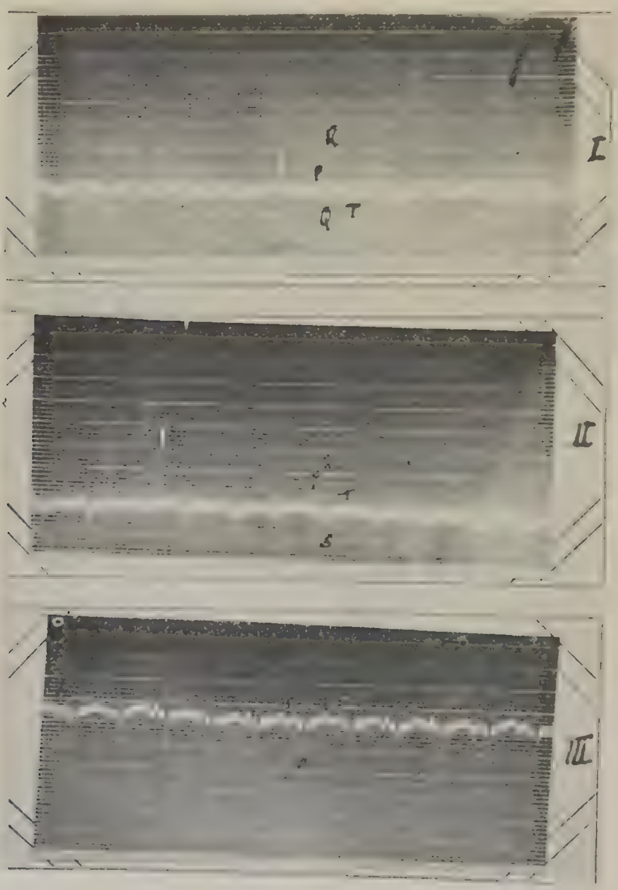
Fig. 4.



Cat 3, forty-eight hours after diphtheria toxin. Deep inversion of T in lead one and left preponderance.

ysis and circulatory failure are often associated, and attempts have been made to explain both on a nervous mechanism. Here we have an instance of paralysis occurring at a time when there is evidence of recovery in the heart.

FIG. 5.



Six days after diphtheria toxin. Partial return to normal.

Figures 3, 4 and 5 illustrate the changes in an experiment.

Cat 3 was electrocardiographed (Fig. 3) on February 22nd and .05 cc. of toxin injected subcutaneously. On February 24th the animal appeared inactive and listless but otherwise seemed normal. Fig. 4 shows the deep inversion of the T wave in lead one and the marked left preponderance which has developed. On February 26th the animal seemed very apathetic and refused its food. On February 28th the general condition was better, but it still refused food. Electrocardiogram on that date (Fig. 5) showed a lesser degree of T wave inversion and less marked left preponderance.

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[1] Rohmer, P., *Z. Exp. Path. u. Therap.*, 1912, xi, 426.
[2] McCulloch, H., *Am. J. Dis. Child.*, 1920, xx, 89.
[3] Smith, S. C., *J. Am. Med. Assn.*, 1921, lxvii, 765.
[4] Marvin, H. M., *Am. J. Dis. Child.*, 1925, xxix, 433.

3393

Work of the Left Ventricle in Normal, Hypertension and Arteriosclerosis.

GEORGE FAHR.

From the Department of Pathology, University of Minnesota.

It is very probable that increased work of any chamber of the heart is followed after some time by hypertrophy and dilation of that chamber. It is of interest to know the exact increase in work for the left ventricle of the heart in hypertension and in generalized arteriosclerosis.

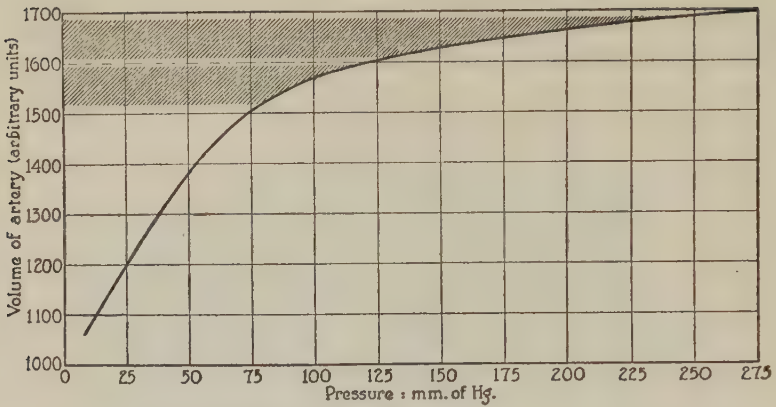
The work of the left ventricle per beat is represented by the equation:

$$\int_{P_1}^{P_2} PdV + \frac{Sv^2}{2g}$$

where P is the pressure of the blood in the aorta, P_1 the diastolic and P_2 the systolic pressures, respectively; V the volume of the arterial system, corresponding to the pressure P, and dV an infinitesimal change in the volume when a small amount of blood is ejected from the left ventricle and the pressure increased by the amount dP. S is the stroke volume, v the velocity of the blood in the aorta and g the constant of gravity. The second term may be neglected in our problem because it makes up only 1 or 2 per cent of the work of the left ventricle and is constant for equal stroke volumes and velocities in the aorta.

If we can find the relation between P and V we can integrate the first term of the work equation and can thus find the work of the left ventricle per beat. Bramwell, Downing and Hill[1] have determined the relation between the volume and pressure in the "mean normal" artery of man. The curve, Fig. 1, is a reproduction of their curve of this relation. If we assume that in the "normal" person the diastolic blood pressure is 80 and the systolic 120, then, ac-

FIG. 1.



Integration of $\int_{P=80}^{P=120} PdV$ and $\int_{P=130}^{P=240} PdV$ from Pressure-Volume relation of Bramwell, Downing and Hill's ''mean normal'' artery curve. 1 scale division along ordinates equals 100 cc. volume increase. 1 scale division along abscissa equals 25 mm. Hg. pressure.

cording to this curve, the corresponding change in volume when the pressure rises from the diastolic to the systolic is 80 cc. The shaded area between 1515 cc. and 1595 cc. and 80 mm. Hg. and 120 mm. Hg. represents the definite integral

$$\int_{P=80}^{P=120} PdV$$

or the work done in forcing 80 cc. of blood into this arterial system between the pressures 80 and 120. I have determined this area by means of a planimeter, and have calculated the work represented by the shaded area by comparison with the area of one of the small squares, each of which represents 34 gram meters of work. The work represented by this shaded area is 98.6 gram meters of work. In other words, 98.6 gram meters of work are performed when 80 cc. of blood are forced into the "mean normal" arterial system between the pressures 80 mm. Hg. and 120 mm. Hg.

If now 80 cc. of blood are forced into the "mean normal" artery between the diastolic pressure of 130 mm. Hg. and the systolic of 240 mm. Hg., the work represented by the upper shaded area is 192.4 gram meters. In other words, 192.4 gram meters of work are done in forcing 80 cc. of blood into the "mean normal" artery between a diastolic pressure of 130 mm. Hg., and a systolic pressure of 240 mm. Hg. The increase in work necessary to force 80 cc. of blood into the "mean normal" arterial system between the pressures

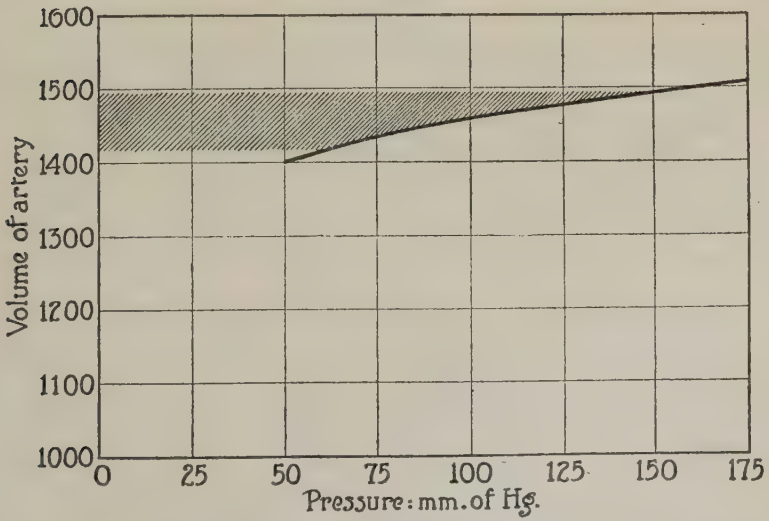
240/130, is 95 per cent greater than the work necessary to force the same amount of blood into this arterial system, between the normal systolic and diastolic pressures 120/80.

In Fig. 2 and in Fig. 3 a piece of the curve of Volume-Pressure relation is drawn for an arterial system in which the rigidity of the system is 2.8 times as great as in the "mean normal" artery of Fig. 1. The rigidity of the arterial systems whose Pressure-Volume relations are shown in Figs. 2 and 3 is 1.7 times as great as the rigidity of the arterial system of a man 80 years old investigated by Bramwell, Hill and McSwiney.[2] We can regard this rigidity as that of advanced arteriosclerosis.

The shaded area in Fig. 2 represents the work done in forcing 80 cc. of blood into this rigid artery between the diastolic pressure 60 mm. Hg. and a systolic of 145 mm. Hg. 105.4 gram meters of work are performed when the stroke volume of 80 cc. is forced into this rigid arterial system between the pressures 60 mm. Hg. and 145 mm. Hg. or 7 per cent more work than is done in forcing the same volume at normal pressures into the "mean normal" artery.

The pressure within the rigid arterial system will rise from 80 mm. Hg. to 185 mm. Hg., if we force 80 cc. of blood into it, when

FIG. 2.



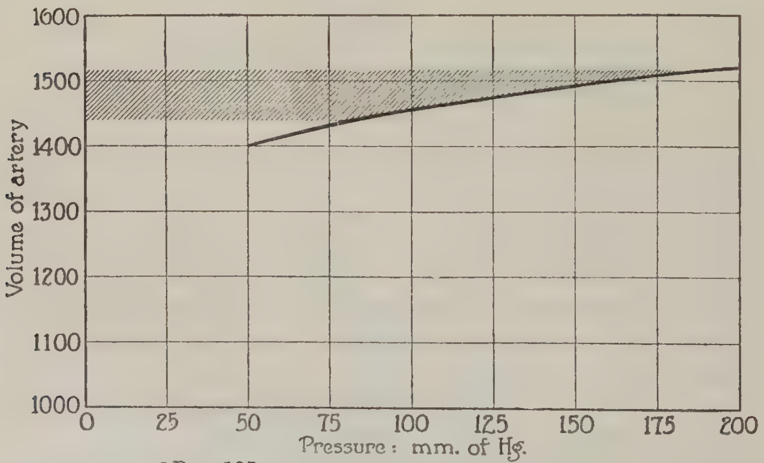
Integration of $\int_{P=60}^{P=145} PdV$ from Pressure Volume relation of an arterial system whose rigidity is 2.8 times that of the "mean normal" artery. The pressure volume curve is the end piece of Bramwell, Downing and Hill's "mean normal" curve shifted into the area of pressures 50-175.

it is already filled to a pressure of 80 mm. Hg. The work done in forcing 80 cc. into this arterial system between a diastolic pressure of 80 mm. Hg. and a systolic of 185 mm. Hg. is represented by the shaded area of Fig. 3, and is equivalent to 136 gram meters of work, or 40 per cent more work than is performed in forcing 80 cc. of blood into the "mean normal" artery at pressures between 80 diastolic and 120 systolic.

Before drawing conclusions from the above determinations, it is necessary to go back and criticise the method by which Bramwell, Downing and Hill obtained the values of the Pressure-Volume relation of their "mean normal" arterial system. In the first place, they determined the data for the coefficient of volume elasticity by measurements of pulse wave velocity at various pressures in isolated carotid arteries obtained soon after death. The volume elasticity of an artery may change after death but Bramwell and Hill[3] have shown that the velocity in an isolated artery compares very well with the velocity measured in a living man. So this objection has little weight insofar as the general form of the curve is concerned.

It is not rigorously correct to apply measurements done on the carotid artery to the pressure-volume relations in the whole arterial system. But insofar as the general problem of calculation of heart

FIG. 3.



Integration of $\int_{P=80}^{P=185} PdV$ from Pressure Volume relation of an arterial system whose rigidity is 2.8 times that of the "mean normal" artery. This pressure volume curve is the end piece of the "mean normal" curve of Fig. 1 shifted into the area of pressures 50-200.

work is concerned under the conditions of this investigation and for the purposes of this study, the determinations of the work of the left ventricle from the Pressure-Volume relations of the "mean normal" artery of Bramwell, Downing and Hill is justifiable.

Bramwell, Downing and Hill constructed their curve of the Pressure-Volume relations in the "mean normal" artery without taking into consideration the "run off" through the capillaries during systole.* The rate of capillary "run off" during cardiac systole increases from a minimum at the beginning of systole to a maximum at the end of cardiac systole. For this reason larger volumes are ejected from the heart in the latter parts of systole when the blood pressures are higher than would be indicated by Bramwell, Downing and Hill's curve.

Our calculation of the work of the heart from Bramwell, Downing and Hill's curve of the Pressure-Volume relation in the "mean normal" artery is somewhat less than the real value of this work, because larger volumes are ejected from the heart at higher pressures toward the end of systole than would be indicated by the Pressure-Volume relations of the curve of the "mean normal" artery. If we correct this curve by the assumption that the systole takes 0.3 seconds and the diastole 0.6 seconds, and that the "run off" from the arteries is proportional to the pressures in the aorta, we calculate an amount of work which is not quite 10 per cent greater than the work calculated from Bramwell, Downing and Hill's curve. The blood pressure in the periphery does not vary as much as it does in the central vessels, and therefore the "run off" is much steadier than that used in the above assumption We are therefore justified in saying that the errors in our calculation are less than 10 per cent, provided the stroke volume remains the same in each calculation. An error of this order vitiates in no way the following general conclusions:

1. In hypertension the work of the left ventricle is enormously increased above the work done at normal pressures, increase in work being very nearly proportional to the increase in the systolic pressure. This increase in work is more nearly proportional to the increase in systolic pressure than it is to the increase in the sum of the systolic and diastolic pressures divided by two, often erroneously called the "mean" pressure. The fact that the "run off" from the

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* Bramwell, J. C., Downing, A. C., and Hill, A. V., were cognizant of this, but felt that the amount of blood ejected through the capillaries during cardiac systole would not alter many of the conclusions to be drawn from the curve as constructed by them without consideration of this "run off."

arterial system is greater during the period of systolic pressure makes this conclusion even more secure.

2. Arteriosclerosis increases the work done by the left ventricle only very little if there is as much fall in diastolic pressure as there is rise in systolic pressure. If the diastolic pressure remains constant and the systolic rises, the work of the left ventricle increases, but it is largely this rise in systolic pressure that increases the work of the heart under these circumstances. In other words, arteriosclerosis only increases the work of the heart markedly insofar as it necessitates increased blood pressure. The hypertension is then largely the cause of the increased work of the left ventricle.

This is a complete report.

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[1] Bramwell, J. C., Downing, A. C., and Hill, A. V., *Heart*, 1923, x, 289.

[2] Bramwell, J. C., Hill, A. V., and McSwiney, B. A., *Heart*, 1913, x, 233.

[3] Bramwell, J. C., and Hill, A. V., *Proc. Roy. Soc. London*, 1922, 93 series B, 298.

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3394

An Immunological Study of the Relation Between Pernicious Anemia and Anemia Due to Welch B. Toxin.

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Chronic anemia was produced in rabbits by the injection of Welch B. toxin. The blood picture was characterized by (1) low color index, (2) anisocytosis without distortion of the shape of the cells, (3) rarity of nucleated red corpuscles. The histological examination of the organs showed changes consistent with blood destruction from any cause. Study of the spinal cord for degenerative changes, such as are frequently found in pernicious anemia, were negative.

The anemia which developed, however, was characterized by remissions when the injections were continued over long periods of time. The blood picture in rabbits which were given non-fatal doses returned to the normal despite the continuance of injection of the toxin. If large doses were then given the resistance could be broken down and anemia once more produced. This phenomenon could be repeated. It seemed, therefore, theoretically possible that the remissions in pernicious anemia were due to a varying immunity on the part of the human organism to the Welch B. toxin, and that, furthermore, the protoplasm of the rabbit was not designed to produce the typical changes of pernicious anemia as it occurred in man. For this reason it seemed important to determine the immunologic reactions associated with anemia due to Welch B. toxin, and compare them to those observed in pernicious anemia.

A study of the anti-hemotoxin furnished a method for making comparison between the clinical and the experimental anemia. As shown by Bull and Pritchett,[1] Welch toxin when mixed with serum containing anti-hemotoxin and injected intravenously into an animal does not result in destruction of red cells, whereas the toxin injected alone causes severe anemia and death. Lyall[2] developed a technique for measuring the hemolytic activity of the toxin *in vitro,* and the titre of the anti-hemotoxic serum used. In this test varying amounts of toxin are mixed with 0.1 cc. of serum, a 5 per cent suspension of red blood cells is added and the mixture incubated for two hours. The absence of hemolysis measures the amount of anti-hemotoxin present in the serum.

The sera of 7 rabbits in whom chronic anemia was produced gave complete inhibition of hemolysis against 0.1 to 0.7 cc. of toxin. Anti-hemotoxin was present both in the periods of severe anemia and in the periods of remission. The first appearance of anti-hemotoxin occurred 3 weeks after the first injection in small amounts and was synchronous with the beginning of an elevation in blood count. The sera of 3 rabbits who were rendered acutely anemic in less than 3 weeks did not inhibit hemolysis. The development of anemia for the second or third time did not appear to be due to the disappearance of anti-hemotoxin, but to the fact that the resistance of the animal was broken down by large doses of toxin.

Eight cases of pernicious anemia, representing various stages of the disease from the severely anemic patient to the patient in remission, were tested. In none was there inhibition of hemolysis. Negative results were also obtained in 9 normal individuals, 3 normal rabbits and 2 patients with severe secondary anemia.

The complete absence of anti-hemotoxin in the pernicious anemia patients studied offers no evidence that this disease has any relation to experimental anemia in the rabbit due to the toxin of the Welch bacillus.

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[1] Bull, C. G., and Pritchett, I. W., *J. Exp. Med.*, 1917, xxvi, 129.

[2] Lyall, H. W., *J. Med. Res.*, 1914, xxx, 515.

3395

The Possible Relationship Between Absorption of B. Welchii Toxin and Pernicious Anemia.*

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New York City.

The experiments reported here are in continuation of our investigation of the possible relationship between the absorption of *B. welchii* toxin from the intestine, and the condition of pernicious anemia. In a previous communication[1] there was described a severe type of anemia produced in monkeys by the intravenous inoculation of small doses of potent *B. welchii* toxin. In all the animals, however, after 3 weeks treatment, an immunity became apparent which was not broken down even by a greatly increased dosage, although abnormalities of erythrocytes and a leucopenia persisted for some time.

It was next attempted by the continued inoculation of dosages of *B. welchii* toxin just below the limit of toleration, to cause such serious injury to the hematopoietic system as might prove irreparable, and to induce a progressive degenerative condition resembling that of pernicious anemia. The method of toxin preparation was essentially as described heretofore, except that somewhat larger pieces of fresh sterile pigeon muscle were added to the casein digest broth. Employing our pernicious anemia *B. welchii* strain, "Navitol", sterile toxins were obtained which were lethal for pigeons within 24 hours after intramuscular inoculation of 0.5 cc. Two young ring-tail monkeys *(Cebus capucinus)* were given a long series of intravenous toxin inoculations in dosage sufficient to keep the animals in a condition of more or less severe anemia for 9 to 10 weeks, in contrast with the 3 or 4 weeks period of the previous experiment. After about 10 weeks, however, in spite of greatly increased dosage, it was not possible to check the tendency of reversion toward normal, and we became convinced that intravenous inoculations of *B. welchii* toxin would not damage the hematopoietic tissues to such a degree that the condition was irreversible.

It was then decided to determine the effect following the introduction of the toxin through the more or less inflamed or injured gastro-intestinal mucous membrane. For, if the absorption of *B. welchii*

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* This investigation has been aided by a fund contributed by Mrs. W. K. Vanderbilt.

TABLE I.

Effect on the Blood of Monkeys of Prolonged Intravenous Inoculations of *B. welchii* Toxin.

Dates	Erythrocyte Count		Hemoglobin Per Cent		Color Index		Anisocytosis		Poikilocytosis		Nucleated Red Cells*		Punctated Basophiles		Leucocyte Count	
1926	J	K	J	K	J	K	J	K	J	K	J	K	J	K	J	K
1/13	4,600,000	4,780,000	86	92	0.93	0.9	— —	— —	— —	— —	—	—	—	—	6,800	6,600
1/15	2,790,000	1,960,000	76	54	1.4	1.4	++	— —	— —	— —	—	—	—	—	10,800	8,800
1/18	4,500,000	1,290,000	85	23	0.94	0.9	+++	+++	— —	— —	2	3	—	—	6,200	4,200
1/19	2,920,000	1,510,000	60	24	1.4	0.8	++++	—	+	— —	1	1	—	—	10,000	4,200
1/21	2,060,000	1,170,000	50	24	1.25	1.0	++++	++++	+++	++	6	—	+	—	11,000	4,600
1/25	2,500,000	2,510,000	68	55	1.36	1.1	++	—	—	+++	2	—	—	—	4,000	4,400
1/26	1,700,000	2,070,000	50	49	1.47	1.2	++++	+++	+	+	22	3	++	—	11,200	8,000
1/28	1,740,000	1,530,000	55	32	1.6	1.0	++++	++++	++	—	3	10	—	—	9,600	7,800
2/3	2,080,000	1,630,000	46	50	1.15	1.59	+	—	+	—	—	—	—	—	4,400	4,400
2/8	2,060,000	2,410,000	63	59	1.57	1.2	++	+++	++	++	—	—	—	—	4,600	6,800
2/18	2,040,000	2,090,000	63	65	1.57	1.6	—	++	—	—	—	—	—	—	6,800	6,600
2/26	2,480,000	2,090,000	66	45	1.5	1.1	—	+	—	—	3	—	—	—	4,600	4,800
3/3	2,530,000	1,940,000	65	58	1.3	1.5	—	+++	+++	++	3	—	—	—	8,600	5,600
3/10	2,510,000	1,990,000	68	67	1.4	1.8	—	—	—	—	—	—	—	—	6,600	7,200
3/19	3,120,000	2,640,000	80	78	1.27	1.5	++	+	++	+++	—	—	—	—	5,600	6,200
4/3	4,090,000	3,190,000	87	61	1.08	0.9	—	—	—	—	—	—	—	—	5,900	4,800

Inoculations and examinations about every three days: Jan. 14-30, 0.5 to 2.5 cc.; Feb. 1-Mar. 1, 3 to 10 cc.; Mar. 1-15, 10 to 20 cc.

*Number per 100 leucocytes counted.

toxin is the factor of primary importance in the etiology of pernicious anemia, it probably gains entrance to the vascular system from this locality.[2] Experiments conducted some time previously had indicated that this toxin introduced into normal intestines of monkeys was not absorbed therefrom, at least not to a degree sufficient to cause pronounced changes in the blood.

About this time the work of Leake and Ritchie[3] came to our attention. They found that the repeated treatment of the stomach of atropinized dogs with 5 per cent sodium fluoride would give rise in time to a condition of atrophic gastritis, with a subsequent complete achlorhydria and pronounced anemia. Their methods, kindly supplied by Dr. Leake, were applied to monkeys in a modified form, with the purpose of increasing the absorption of *B. welchii* toxin from the gastro-intestinal tract. At weekly intervals 3 monkeys: M, O and P, ½ hour after an intramuscular injection of ⅛ mg. of atropin, were fed 8 cc. of 1 to a 3 per cent aqueous solution of sodium fluoride through a stomach tube. A 5 per cent strength, tried at first, proved fatal to the animal. The fluoride was allowed to remain in contact for 7 minutes, during which time the animal was rolled about to insure contact with all surfaces of the stomach. Then 15 cc. of a 1 per cent aqueous solution of calcium carbonate was introduced through the tube, to precipitate the fluoride and finally, as much as possible of the stomach content was aspirated. Complete emptying generally resulted. In addition, 2 days following the fluoride treatment, monkeys M and O were fed weekly by stomach tube, 20 cc. of a whole fluid culture of *B. welchii* (Navitol strain) prepared as described heretofore. Monkey P served as a control for the fluoride alone.

It was found that 3 per cent sodium fluoride was about the maximal strength which could be used safely, and after one or two treatments the dosage was reduced to 1 per cent. The fluoride caused an acute catarrhal inflammation of the gastric mucosa, which tended to subside in 24 to 48 hours. The pathological changes following long continued treatment are described later and the details of the treatment and its effects on the blood are reported in Table II.

As indicated, the 2 monkeys treated with the fluoride, and also the toxin died, 1 of them (O) after 60 days and the other, (M) after 103 days, both of them showing an extreme degree of anemia. The control (P), however, was alive and in good condition after 115 days when it was chloroformed, 39 days after the fluoride treatments had been stopped. The control showed a loss of weight of 8.7 per cent (996 to 875 grams) as contrasted 23.3 per cent (1356 to 1040 grams) for monkey M. About 8 weeks after the start of

TABLE II.

Effect of Absorption of *B. welchii* Toxin from the Gastro-intestinal Tract on the Blood of Monkeys.

M and O, Toxin treated. P, Control.

Dates of blood exam.	Erythrocyte Count in Millions			Hemoglobin Per Cent			Color Index			Leucocyte Count in Thousands			Anisocytosis			Poikilocytosis			Nucleated Red Cells per 100 leucocytes		
	M	O	P	M	O	P	M	O	P	M	O	P	M	O	P	M	O	P	M	O	P
Normal 4/1	4.61	4.94	4.99	89	92	96	0.96	0.93	0.97	10.0	8.6	7.4	—	—	—	—	—	—	0	0	0
4/20	2.36	2.36	2.96	68	67	71	1.5	1.5	1.2	11.0	9.0	11.2	++	++	+	– –	– –	– –	0	0	0
4/26	2.75	2.68	3.90	63	70	74	1.1	1.3	0.94	5.2	9.6	9.0	++	+++	+	– –	– –	– –	0	0	0
5/3	2.93	2.78	3.76	67	69	78	1.1	1.2	1.0	9.6	10.0	9.2	+	+	– –	– –	– –	– –	0	0	0
5/10	2.39	2.73	3.96	65	64	76	1.4	1.2	0.97	8.8	8.0	10.8	+	+	– –	+	– –	– –	1	0	0
5/17	2.00	1.65	3.09	55	55	67	1.3	1.7	1.0	5.8	5.6	8.2	+	++	– –	+	++	– –	0	1	0
5/24	1.61	1.86	4.07	53	50	75	1.6	1.3	0.93	7.2	8.2	9.2	+	++	+	– –	– –	– –	0	0	0
6/2	3.17	2.73	3.52	70	55	62	1.0	1.0	0.88	8.0	10.0	7.6	++	+++	+	++	– –	– –	0	0	0
6/9	2.45	1.97	3.05	59	53	72	1.2	1.3	1.2	5.6	8.8	8.2	+	+++	– –	– –	++	– –	0	0	0
6/16	1.51	Died 6/10	2.43	33		65	1.1		1.3	4.6	—	8.0	+		++	– – –		– –	6	0	0
6/23	1.29	—	3.00	35		70	1.0		1.2	3.2	—	7.8	+++		+	++		– –	1		0
6/30	0.91	—	2.78	10		62	0.55		1.1	3.2	—	6.2	++++		– –	+		– –	4		0
	Died 7/2	—	—																		
7/8	—	—	2.95			62			1.0			6.4			+			– –			0
7/14	—	—	2.04			70			1.7			8.8			+ –			– –			0
7/21	—	—	3.16			65			1.0			6.2			+ –			– –			0

Sodium fluoride treatments:
M, total 9, March 31 to June 12, 1926.
O, total 8, April 6 to June 9, 1926.
P, total 11, April 5 to June 14, 1926.

B. welchii toxin treatments:
M, total 13, April 1 to June 24, 1926.
O, total 8, April 20 to June 4, 1926.
P, control, no *B. welchii* toxin.

the treatments, the reaction of the gastric contents 3 hours after a meal was taken. All 3 monkeys showed a H-ion reading of about 5.0, as contrasted with 4.6 for 2 untreated monkeys on the same diet. Thus at this state, at least, the fluoride had not caused any notable decrease in gastric acidity.

The blood examination data in Table II presents very definite evidence for the absorption of *B. welchii* toxin. This is indicated not alone by the lower erythrocyte count, the Hb per cent and higher color index for M and O than for P, but also in the much more marked degree of anisocytosis, and at times, of poikilocytosis in the 2 former animals at nearly every examination. Particularly striking are the differences revealed at the May 24th examination, when the blood condition of the control P was nearly normal, whereas M and O both showed severe anemia.

It is of interest, too, that an immunity did not develop when the toxin was administered under these conditions. Monkey O died about 8 hours after a treatment with 3 per cent sodium fluoride, but M died 20 days after the fluoride, but not the toxin treatments, had been stopped. This finding may be contrasted with the immunity regularly developing during intravenous inoculations. Whether or not this difference was due solely to the fluoride factor has not as yet been determined.

Monkey M, about 2 weeks before death, passed into a profound state of anemia, showing an erythrocyte count of 1,510,000 hemoglobin 33, and a marked leucopenia.

At necropsy all the organs of M showed an extreme degree of anemia but no marked lesions. The spleen and retroperitoneal lymph nodes were not enlarged. The bones were dead white and brittle, and the marrows softer and lighter red than is normal for these monkeys. On the other hand, the organs of P were only moderately anemic, and the bone marrows were a much darker red than in the case of M.

The microscopical findings, as reported by Dr. James Ewing, proved of particular interest. The stomach of the control P monkey revealed evidence of a mild catarrhal gastritis with considerable lymphocyte infiltration of the mucosa, but the glands and acid cells stained well and appeared normal. In contrast the stomach of M exhibited a very severe catarrhal gastritis, desquamation of the acid cells, and well marked lymphocyte infiltrations of the submucosa. No glands with intact secreting cells were noted. The greater interest, however, pertains to the microscopical differences in the marrows. Comparing the femur marrows of the 3 monkeys the findings were as follows: That of control P was normal in appearance, being solidly packed with normal marrow cells in normal relations. That of O appeared very cellular and congested, mostly large and small lymphocytes, islands of nomoblasts missing but a good many nucleated hemoglobin-holding cells which are much larger than usual; no evidence of blood destruction. The marrow of M, the monkey which had received 5 more toxin feedings than O, appeared very cellular. The rather numerous fat cells are undergoing mucinous degeneration and their envelopes are often broken. There is a moderate deposit of recent blood pigment in the macrophages. No islands of nucleated reds are seen, but there are many scattered nucleated reds, staining poorly. The impression is gained of a severe toxic degeneration of the marrow tissue affecting fat cells and erythrocytes, both with and without nuclei. Unfortunately for the purposes of this comparison the control P was not killed until 5½ weeks following the last sodium fluoride treatment. The blood condition, however, had remained much the same and it is not believed that the marrow findings would have been materially different at an earlier examination.

These tissue findings, as well as those for the blood, present definite evidence of the absorption of *B. welchii* toxin through the sodium fluoride injured gastro-intestinal mucosa, with resulting pathological changes in the blood and tissues markedly over and above that caused by fluoride alone. The failure of these animals to develop a resistance to the toxin, as has invariably followed intravenous inoculation, may have been due to the depressing influence of the chemical, or possibly to the portal entry of the toxin. Further study is being given of this mode of treatment on antitoxin production. This is a complete report.

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[2] Moench, L. M., Kahn, M. C., and Torrey, J. C., *J. Infect. Dis.*, 1925, xxxvii, 161.

[3] Leake, C. D., and Ritchie, G., *Am. J. Physiol.*, 1926, lxxvi, 234.

3396

The Anamnestic Reaction: Response of Previously Immunized Animals to Heterologous Antigens.

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Cole[1] observed, in 1904, that after an animal has been immunized subsequent injection of the same antigen causes antibodies to appear earlier than after the first injection. Previously immunized animals also show circulating antibodies after the injection of amounts of homologous antigen which, in animals injected for the first time, are insufficient to cause the appearance of demonstrable antibodies. A clinical counterpart of these experiments has been observed in what is called accelerated serum disease: symptoms and circulating precipitin appear earlier when a patient is treated a second time with serum. It has been observed,[2] too, that individuals previously vaccinated against typhoid may show a reappearance of typhoid agglutinins during the course of typhus. Bieling[3] studied this anamnestic phenomenon in rabbits, using as antigens the Shiga and His-Russel types of *B. dysenteriae* and *B. typhosus.* He found that the heterologous antigen would call back, so to speak, the agglutinins for the antigen previously administered.

In subjecting the anamnestic reaction to a systematic study we have used 4 antigens sufficiently remote from each other in biological origin to exclude group reactions. A series of 18 rabbits has been studied over a period of 3½ years. The antigens used were horse serum, chicken serum, crystalline egg albumin and *B. typhosus.* Immunization with horse serum and chicken serum was accomplished by giving 3 intravenous injections (0.5 cc., 1.0 cc., 1.5 cc.) at intervals of 5 days. For the egg albumin immunization, 3 intravenous injections (1.0 cc., 2.0 cc., 3.0 cc.) of a 3 per cent solution at 5 day intervals were given. Suspensions of *B. typhosus* at first heat killed, and later, living organisms were injected intravenously 7 times in the course of 16 days, the number of organisms amounting to approximately 5½ billion. Titrations of precipitin or agglutinin were made 5 days after the last antigen injection, and then at intervals of 7 days. Reinjection with a second antigen was not made until the antibodies produced during the previous immunization had disappeared entirely from the circulation. It was found that rabbits previously injected with horse serum would frequently show a reappearance of horse serum precipitin when subsequently given injections of chicken serum. Immunization later with egg albumin sometimes caused not only the appearance of precipitin for egg albumin but also in some cases the reappearance of both horse and chicken precipitin. A later immunization with *B. typhosus* caused the appearance in the circulation of *B. typhosus* precipitin, and precipitin for one or more of the antigens which had previously been injected. If, after immunization with the fourth antigen, only one heterologous precipitin reappeared, it was always the one for the last antigen used; if two heterologous antibodies appeared, they corresponded always to the last two antigens used. In a few instances heterologous antibodies for all three of the antigens previously used reappeared in the circulation when the fourth antigen was injected. Efforts to cause this anamnestic appearance of antibodies by the injection of such non-antigenic substances as 10 per cent NaCl solution, peptone, and turpentine (subcutaneously) yielded only negative results.

The anamnestic reaction is not satisfactorily explained on the basis of heterogenetic antigens; nor does it harmonize with the Ehrlich theory. This is a preliminary report.

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3397

Effect of Ligation of the Pancreatico-Duodenal Artery on the Blood Sugar and Urine.

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In previous papers[1, 2] the author and co-workers presented experimental evidence which seemed to indicate that changes in the permeability of the capillary vessels of the pancreas, produced by perfusions of its arterial supply, resulted in a mobilization of trypsin into the blood stream, via the portal circulation, with the production of a hyperglycemia and glycosuria. It was also suggested that the presence of trypsin in the blood stream may be part of the mechanism regulating the secretion and activity of insulin under normal and pathological conditions.

In part our work has received confirmation by the histological findings of Horning,[3] Collens,[4] however, reaches certain conclusions which are not in accord with our own. This investigator employed a technic totally different from ours, and failed to ascertain what differences in results his procedures would cause.

The purpose of this communication is to record additional data which throws considerable light on the subject.

We perfused the pancreatico-duodenal artery *without the application of ligatures to adjacent vessels,* whereas Collens perfused the hepatic artery *after ligating* the pancreatico-duodenal. Then he perfused the pancreatico-duodenal artery *after ligating* the hepatic. No mention is made of control experiments on the effect of ligation of these respective vessels without the perfusion of any others.

The result of ligation of the hepatic arteries upon carbohydrate metabolism is known to be comparable in its effect to complete ablation of the liver.[5, 6] It has been termed "Bloodless hepatectomy,"

PROTOCOL 1. Female cat, weight 3.4 kgm. Amytal anesthesia.*
Laparotomy; ligation of pancreatico-duodenal artery. Abdomen closed in two layers.

Observations.

Date	Time	Blood Sugar†	Remarks.
2/18/26	11:30 a. m.	0.142%	
	11:45		Pancreatico-duodenal artery ligated.
	12:25 p. m.	0.294%	
	4:00 p. m.	0.186%	
	10:00 p. m.	0.164%	
2/19/26	12:15 p. m.	0.210%	Urine removed from bladder contained 1.25% sugar.

* In view of the existing controversy as to the effect of amytal upon the blood sugar, control experiments were made and no hyperglycemia was observed.

† Blood sugar estimations were made by the Folin-Wu method.

PROTOCOL 2. Male cat, weight 3 kgm. Amytal anesthesia.
Laparotomy; Temporary ligation of the pancreatico-duodenal artery; ligature left in place 9 minutes, released and abdomen closed in two layers.

Observations.

Date	Time	Blood Sugar	Remarks.
3/30/26	11:54 a. m.	0.140%	
	11:02		Ligature applied to pancreatico-duodenal artery.
	11:11		Ligature released.
	11:50	0.276%	
	3:26 p. m.	0.154%	
4/1/26			Urine contained 0.25% sugar.

and is accompanied by a rapid disappearance of glycogen from the liver and a hypoglycemia. In view of the profound effects upon the carbohydrate metabolism produced by the ligation of the hepatic arteries, such a procedure cannot be employed with safety for the purpose of studying the effects of perfusion of the pancreas.

On the other hand, the effect of the ligation of the pancreatico-duodenal vessel is not so well known. I therefore wish to record the influence of such a procedure upon carbohydrate metabolism.

These protocols are typical of the reaction which follows the ligation of the pancreatico-duodenal artery, as determined on 8 cats. Constriction of the vessels for a few minutes causes a hyperglycemia which in most instances is accompanied by a glycosuria.

It is clear, therefore, that the effect which Collens ascribes to the perfusion of the hepatic artery after ligation of the pancreatico-duodenal artery, can at present be interpreted only as the result of the ligation of the pancreatico-duodenal artery.

Summary. 1. Application of a ligature to the pancreatico-duodenal artery produces *per se* a marked hyperglycemia and glycosuria. 2. The hyperglycemia and glycosuria following ligation of the pancreatico-duodenal artery and perfusion of the hepatic cannot be interpreted as resulting from the latter procedure alone. This is a preliminary report.

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[4] Collens, W. S., *J. Biol. Chem.*, 1925, lxiv, 461.

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3398

Acetylation.

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Acetylation is a process sometimes used by the body as a method of detoxication. When, for example, *p*-amino-benzoic acid is fed either to man or to rabbit, it is partly excreted as *p*-acetyl-amino-benzoic acid. It has seemed to us that this reaction might find an application in studies dealing with intermediary metabolism. That acetaldehyde and acetic acid are two products formed in the degradation of foodstuffs is generally believed. The evidence for their formation, like the evidence for the formation of other substances which are supposed to represent the intermediary steps in the metabolism of foodstuffs, is, as a rule, indirect rather than direct. By using such a substance as *p*-amino-benzoic acid, we are enabled to *fix,* as it were, the acetyl group. Since the acetylated compound formed can be recovered in the urine, and its amount determined, a measure of the extent to which acetylation occurs in the body may be obtained.

An important question now arises, to what extent will acetylation be affected if, in addition to the *p*-amino-benzoic acid, we feed the rabbit various substances which we have reason to believe influence the amount of acetaldehyde or acetic acid formed in the body? The results might give us more direct, more concrete evidence as to whether substances do or do not pass through the acetic aldehyde-acetic acid stage in the course of their degradation in the body.

Using rabbits as our test animals, we first fed them *p*-amino-benzoic acid for 4 days, then, in addition, the substance under examination for 4 days, and, finally, the *p*-amino-benzoic acid alone for 4 more days. The 24 hour samples of urine were collected, and the acetylated compound recovered and weighed. The percentage increase in the acetylated product as a result of the addition to the diet of the substance under examination, could now be calculated. The results are as follows, the numbers representing the percentage increases: alcohol 207, tyrosine 188, ethyl ester of acetoacetic acid 176, sodium acetate 158, β-oxy-butyric acid 133, pyruvic acid 126, glyceric aldehyde 118, histidine 102, glycerol 93, acetic aldehyde 81, olive oil 52, serine 49, *n*-leucine 42, fructose 40, glycocoll 28, alanine 27, lactic acid 22, glucose 16, tryptophane 15, isoleucine 12, sucrose 0.

The results are not altogether in accordance with views generally held. We would have anticipated, for example, that alanine, lactic acid, and even glucose, would be high up on the list; and that acetic aldehyde would not fall so considerably below sodium acetate. We realize, of course, that the numbers given represent quantitative differences to an approximate extent only; but the difference between pyruvic acid and lactic acid, for example, or even between sodium acetate and acetic aldehyde, is so considerable, that experimental errors involved in the method used cannot entirely explain the results. We, therefore, question whether the generally accepted views regarding intermediary metabolism are not, in a number of instances, now open to revision. This is a preliminary report.

3399

The Distribution of Electrolytes in Dogs Following Ligation of Both Ureters.

DANA W. ATCHLEY AND ETHEL M. BENEDICT.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital.

A study has been made of the electrolyte distribution in the blood of 5 dogs with experimental uremia, produced by ligation of the ureters. A comparison of the blood removed before operation and just before death showed very striking changes in the electrolytes. These alterations for serum are averaged as follows:

Total base = +0.1 m. Eq.
Protein anion = —1.1 m. Eq.

Chloride	= —17.9 m. Eq.	Phosphate	= + 9.4 m. Eq.
Carbonate	= — 5.5 m. Eq.	Sulphate	= +12.9 m. Eq.
Total	= —23.4 m. Eq.	Total	= +22.3 m. Eq.

There is an equimolar supplanting of chloride and carbonate by phosphate and sulphate with no consistent change in total base. Analysis of skin, muscle and brain for chloride before and after operation indicated that these tissues tend to follow the changes in the blood. Of 7 pairs of analyses (1 on brain, 3 on muscle and 3 on skin) only one failed to show a decrease in chloride after the onset of uremia. Vomitus contained insignificant amounts of chloride. The determinations on whole blood showed that the cell electrolytes vary in parallel fashion to those of the serum.

The acidosis of uremia in dogs with renal insufficiency is caused by retention of sulphate and phosphate. The base available for these acids is supplied by carbonate and chloride. It is important to note that in average figures chloride supplies three times as much base as carbonate. This is a preliminary report.

3400

Does Methylene Blue Penetrate Into Living Cells?

MARIAN IRWIN.

From the Laboratories of the Rockefeller Institute for Medical Research.

Experiments with dyes tend to favor the theory that the penetration of a basic dye into living cells depends on the fact that the dye has an apparent dissociation constant, and that in the form of free base, which predominates at higher pH values, it penetrates very readily, while in the form of salt it penetrates so slightly that its entrance may be neglected.

According to this theory, methylene blue, which behaves like a strong base,[1] should not penetrate, since it exists in the form of a salt. But the literature is full of statements that living cells are stained by methylene blue. No one, however, has heretofore tested the dye which has actually entered a living cell from a solution of methylene blue. Until this is done, we are in no position to conclude that the dye found inside a living cell is methylene blue, rather than one of its lower homologues (such as trimethyl thionin or "Azure B", which is less basic), since it is very difficult[2] to obtain methylene blue absolutely free from such dyes (especially at higher pH values where some of it changes very readily to the other forms).

Previously, the writer[3] has found that unless there was injury or contamination of the sap from the deeply stained cell wall, the vacuole of the living cell of *Valonia* took up practically no dye[4] from methylene blue dissolved in sea water at pH 5.4, while it took up more at pH 9. Chloroform behaved similarly, in that the higher the pH value of the aqueous solution, the more dye was taken up by the chloroform. The dye which had been taken up by the chloroform was dissolved in sea water after removal from chloroform, and was found to enter the vacuole of living *Valonia* much more readily than was the case with the dye from the methylene blue solution at the same concentration and pH value. The higher the pH value the more rapid was its rate of penetration.

It may be asked whether the dye found in the vacuole and in the chloroform is methylene blue or its homologues. To test this point a series of absorption curves were obtained by spectrophotometric analysis and the following results were obtained:

(1) Absorption curves showed that the sample of methylene blue employed, dissolved either in sea water at pH 9 or in sap of *Valonia* or in artificial sap of *Valonia*, was chiefly methylene blue.

Contamination by other dyes was insufficient to be detected by this method.

(2) The sap of *Valonia* collected from the living cells which had been placed for 1 hour in methylene blue dissolved in sea water at pH 9, contained a dye which was found to be chiefly trimethyl thionin. If methylene blue penetrated it was not present in detectable amount.

(3) The dye, which had been taken up by chloroform from a solution of methylene blue at pH 9, and which was then removed from the latter by shaking up with distilled water, was also found to be chiefly trimethyl thionin.. No detectable amount of methylene blue was present.

(4) The dye, which had been absorbed by the cell wall from a solution of methylene blue at pH 9, and which was then allowed to diffuse out into artificial sap, was found to be chiefly methylene blue. Other dyes were not present in sufficient quantity to be detected.

It may be added here that the cell wall is also capable of taking up trimethyl thionin.

That the methylene blue or other dyes were not present in the vacuole in their reduced forms was proved by the fact that no increase in the coloration of the sap took place on exposing the sap, which was made alkaline, to air.

The experiments were repeated with a fresh water alga, *Nitella** *flexilis,* and approximately the same results† were obtained.

The fact that no methylene blue can be detected in the vacuole of these cells does not prove that it does not enter the protoplasm, since it might not be able to enter the vacuole. We cannot be certain of this point unless we can prove (1) that there is some methylene blue inside the vacuole or inside the cytoplasm, or (2) that if there is any dye in the cytoplasm it is not methylene blue but a homologue.

The experimental results presented in this paper serve to point out the danger in drawing any theoretical conclusion regarding oxidation reduction potential or permeability based on the penetration of the dye from the methylene blue into the living cells.

The writer wishes to acknowledge her indebtedness to Dr. W. C. Holmes of the Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C., for his extreme kindness and skill in making all the spectrophotometric analyses mentioned in this paper. This is a preliminary report.

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* The higher the pH value of the trimethyl thionin, the less rapidly is the cell wall of living *Nitella* stained, while the rate of staining with methylene blue does not seem to change appreciably with varying pH values between pH 5 and pH 9.

† These results are complicated by a greater possibility of contamination of the sap from stained cell wall.

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[1] For a discussion of the apparent dissociation constant of methylene blue *cf.* Clark, W. M., Cohen, B., and Gibbs, H. D., *Public Health Reports*, 1925, xl, 1131.

[2] Clark, W. M., and his collaborators (see footnote 1) state that methylene blue is difficult to purify. It is stated that another dye in the methylene blue may be active in staining (Scott, R. E., and French, R. W., *The Military Surgeon*, 1924, August, p. 1). The writer has tried to obtain methylene blue free from other dyes by repeated extraction with chloroform but at pH 9 it was impossible to obtain such a sample owing to the fact that a certain amount is being converted to another form at such a pH value.

[3] Irwin, M., *J. Gen. Physiol.*, 1925-26, ix, 561.

[4] This result is contrary to the result obtained by Brooks, M. M., *Am. J. Physiol.*, 1926, lxxvi, 360.

3401

Action of Narcotics on the Ameba by Means of Microinjection and Immersion.

STANISLAW HILLER.* (Introduced by Robert Chambers.)

From Cornell University Medical College, New York City.

Comparative studies were made on the influence of the narcotics: ethyl alcohol, chloretone, ether, and chloroform on the protoplasm of *Amoeba dubia,* with special reference to their action on the plasmalemma.

Immersion Experiments: Very weak concentrations (subnarcotic) cause the Amebae to spread out and continue their movements in an expanded condition. This may be an effect of lowered surface tension. Ether in its narcotic concentration, approximately 2 per cent, produces a reversible gelation accompanied by the cessation of all movements. Lethal concentrations of all the narcotics cause the Ameba to round up followed by a sinking of its granules and a disintegration of the plasmalemma.

Injection Experiments: No narcotic effect was observed by injection into the interior of the Ameba. Chloretone in all concentrations increases the fluidity and streaming movements of the interior. Eighty per cent alcohol produces a coagulation which is localized and reversible, when small amounts are injected, and irreversible with large amounts. Pure chloroform and ether, when injected into the cytoplasm, form spherical drops which go into solution more rapidly than when injected into water. The protoplasm is irreversibly coagulated in the environment of the dissolving droplet. Chloroform is more potent than ether in causing coagulation. The irreversible coagula produced by the alcohol, chloroform and ether are pinched off by the living portion of the Ameba.

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* Fellow International Education Board, Rockefeller Foundation.

Local Application of the Narcotic on the Exterior of the Ameba: With saturated chloretone solution and 95 per cent alcohol no local effects were obtained. All the pseudopodia are withdrawn and the Ameba was converted into an actively moving limax form.

Concentrated, aqueous solution of ether produces a local blister on the plasmalemma with a coagulation of the cytoplasm at the base of the blister. A drop of pure ether dissolved the plasmalemma, on remaining in contact with it for five seconds or more. This occurs most rapidly at the tip of a newly forming pseudopodium. As the ether droplet goes into solution there appears in its place a wrinkled pellicle enclosing several highly refractive globules. Both the pellicle and the globules are insoluble in water and in cold 95 per cent alcohol.

Chloroform similarly but more rapidly dissolves the plasmalemma. A small drop on touching the plasmalemma quickly passes through it and the endoplasm in its vicinity becomes coagulated.

An interesting phenomenon, which possibly relates to the problem of pseudopodial formation, occurs when chloroform is allowed to diffuse against the Ameba from the tip of the micropipette. A local elevation of the pellicle takes place, into which the granular endoplasm flows. As the pipette is gradually withdrawn the incipient pseudopodium enlarges and extends in the direction of the moving pipette. In this way the entire Ameba can be made to move, to all appearances, in a normal fashion along any path taken by the tip of the pipette. This is a preliminary report.

I wish to thank Dr. Robert Chambers most sincerely for his kind help in this work.

3402

Cutaneous Reactions with Streptococcus Filtrates in Rabbits Rendered Allergic with Extracts of Guinea Pig Kidneys.

FRANKLIN R. STEVENS AND A. R. DOCHEZ.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital.

In a series of articles published in *La Pediatria*, Di Cristina, Sindoni, and Caronia[1] describe a small diplococcus which they are able to obtain in culture from scarlet fever. These authors attribute certain specific reactions to this diplococcus which resemble the reactions obtained with the hemolytic streptococcus isolated from cases of scarlet fever in this country. The diplococcus has been obtained in blood cultures from scarlet fever and grown in media containing fragments of guinea pig tissue. When the filtrates of these cultures are injected intracutaneously, the response is apparently identical with that resulting from an intracutaneous inoculation with a filtrate of *Streptococcus scarlatinae*. These authors have prepared vaccines which they believe are capable of inducing an active immunity in persons susceptible to scarlatina.

In 1925 Brokman[2] and his associates undertook a comparison of the properties of scarlatinal hemolytic streptococcus and this diplococcus of Di Cristina and Caronia. Carbolized vaccines prepared with whole cultures of the diplococcus were employed to immunize both rabbits and human subjects. In both instances serum obtained after immunization agglutinated the hemolytic streptococcus in low titre. The rabbit serum neutralized the cutaneous reaction obtained with the streptococcus filtrates. As a control experiment, rabbits were immunized with the uninoculated Tarozzi-Noguchi medium employed in culturing the diplococcus for the vaccines. Autolyzed guinea pig tissue was contained in one type of medium and not in the other. Serum from the rabbits injected with the first type of medium agglutinated the hemolytic streptococcus, and neutralized the toxic effects of intracutaneously injected filtrates. Several volunteers, showing positive cutaneous scarlatinal tests, were negative after immunization with this uninoculated broth. The broth without guinea tissue was impotent. The authors conclude that guinea pig kidney and the hemolytic streptococcus associated with scarlet fever contain related antigens.

As we have been interested in sensitization with filtrates of *Streptococcus scarlatinae*, we undertook a study of the reactions occurring during intravenous immunization with autolyzed guinea pig kidney. The kidney was removed and hashed under sterile precautions, then allowed to autolyze at 37° C. in physiologic saline solution. This solution was passed through a Berkfeld filter. Four rabbits were then tested with two extracts of guinea pig kidneys, and with filtrates of streptococci from scarlet fever and erysipelas. One of these rabbits gave faint cutaneous reactions with both streptococcal filtrates. All other initial tests were negative. These animals were given four intravenous inoculations with one of the solutions of autolyzed kidney at intervals of about 12 days. Before each intravenous injection serum was obtained for agglutination and precipitin reactions, and the skin of each rabbit was tested with small amounts (1/10 cc.) of the two kidney extracts, and with similar amounts of scarlatinal and erysipelas filtrates. Tests were made with both heated (98° C. for two hours) and unheated filtrate.

The sera obtained from these rabbits were tested for agglutinins with two strains of streptococcus from scarlet fever, and two from erysipelas, but no agglutinins could be demonstrated. There was no precipitation with the filtrates from the cultures of these strains but the sera from two of the rabbits gave weak precipitin reactions with both kidney filtrates. Precipitation was apparent with a 0.1 dilution of the antigen 11 days after the initial inoculation and with a 0.01 dilution with sera obtained at later intervals.

The rabbits showing the positive precipitin tests were likewise cutaneously sensitive to the filtrates of the streptococcus cultures, and to the kidney extracts. One of the rabbits reacted to the unheated scarlatinal and erysipelas filtrates on the 11th day, but these were the only positive reactions obtained in this animal at any time thoughout the experiment. The second animal was sensitive to the unheated streptococcal filtrates by the 10th day, to both kidney extracts by the 20th day and to the heated filtrates at the end of 4 weeks. All these positive reactions persisted in this rabbit until the 6th week, when the animal was shocked by an intravenous injection of the kidney extract. Following the shock all the reactions were negative. During the course of the experiments controls were made with broth and with 2 atoxic *staphylococcus aureus* filtrates. These injections gave no cutaneous reactions. With the exception of the fact that the reactions with the streptococcus filtrates were at no time neutralized by either scarlatinal or erysipelas antitoxic sera the reactions resembled those obtained in rabbits rendered allergic with filtrates of scarlatinal and erysipelas strains.

The question of sensitization to bacteria is exceedingly complex. It seems unwise to draw too concrete conclusions from experimental work along these lines. This is especially true in the present experiments on account of the limited number of animals which we were able to study. We may say, however, that rabbits can be rendered allergic to certain streptococcal filtrates by injections of autolyzed guinea pig kidney solution, and that the cutaneous reactions in these rabbits show no specificity for either the scarlatinal or erysipelas groups. The reactions are probably not due to toxin, since reactions are obtained with filtrates which have presumably been heated until atoxic. None of the rabbits studied reacted with broth or with *staphylococcus aureus* filtrates. The reactions with the streptococcus filtrates were always the earliest to appear and were the most prominent of all. They appeared simultaneously with the occurrence of the kidney precipitin. For these reasons we must assume that the filtrates and the kidney solutions contain related allergens possessing some degree of mutual specificity. This is a complete report.

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[1] Caronia, G., and Sindoni, M., *La Pediatria,* 1923, xxxi, 14. Di Cristina, G., and Caronia, G., 1925, i, 1. Caronia, G., *La Pediatria,* 1925, xxxiii, 7.

[2] Brokman, H., Fejgin, B., Hirszfeld, H., Meyzner, M., and Przesmycki, F., *Compt. rend. Soc. d. Biol.,* 1925, xciii, 946; *ibid,* 944.

3403

The Reaction of Vesicular Stomatitis Virus to Ultra Violet Light.

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From the Laboratories of the Rockefeller Institute for Medical Research.

Since bacteriological methods have so far failed to yield direct evidence of the nature of the active agent of vesicular stomatitis of horses, which is classed as a filterable virus disease, we have sought indirect evidence of its character in a comparison of the behavior of this virus with that of a common bacterium, *S. aureus,* under exposure to ultra violet light. The reactions of *S. aureus* to measured amounts of monochromatic energy at various frequencies may be considered typical of the behavior of bacterial protoplasms, and we have used the loss of transmissibility of a fixed vesicular stomatitis virus, active for guinea pigs, and the failure of subsequent colony formation by *S. aureus,* as indices of a similar reaction under ultra violet irradiation.

Active vesicle contents from lesions in the posterior foot pads of guinea pigs was aspirated and diluted 1:10 in buffered peptone broth at pH 7.4. This fluid, or a suspension of an 18-hour culture of *S. aureus* in the same medium, was spread on the surface of nutrient agar in small Petri plates, and corresponding areas of the respective plates were exposed to measured monochromatic radiations of the quartz mercury arc. After exposure the bacterial plates were incubated and the resulting colonies in the exposed areas were counted, in comparison with those in like areas from the unexposed portions of the plates. Exposed areas and unexposed control areas were cut from the agar of the virus plates, ground up in phosphate buffer solution at pH 7.5, and 0.4 cc. specimens of the virus-agar suspensions were injected intradermally into the posterior pads of guinea pigs. These animals were subsequently examined for the characteristic lesions of vesicular stomatitis, and the diagnoses were later confirmed by immunity tests.

TABLE I.

λ	Incident energy (ergs per mm.^{2})	*S. aureus* killed per cent.	Vesicular stomatitis virus. Number and reaction of guinea pigs injected with	
			Exposed virus-agar	Unexposed virus-agar (control)
2675	256-270	87-97	3—, 1+	
	512-540	100	5—	5+
3022	23,300—29,900	97—100	4—	4+
3126	32,200—60,400	No visible effect	3+, 1—	4+

The table shows the results of our experiments. λ 2675 is highly bactericidal and shows a corresponding effect on the transmissibility of vesicular stomatitis virus. λ 3022 is within the bactericidal region of the spectrum, though large incident energies are required, corresponding to a relatively low coefficient of absorption of this wave length by bacterial protoplasm. These large incident energies cause a loss of virus transmissibility also. λ 3126 is on the border line of bactericidal ultra violet radiations, and has a questionable effect on the virus. Thus a suggestive similarity is seen in the effects of ultra violet light on the transmissibility of vesicular stomatitis virus and on the death or survival of *S. aureus*. Since the absorption of specific energies is one index of chemical character, and in this instance results in similar effects, these parallel reactions are indirect but suggestive evidence that the substance of the virus is similar in character and chemical constitution to bacterial protoplasm.

3404

Bactericidal Function of the Liver.

BENJAMIN N. BERG, Z. D. ZAU AND JAMES W. JOBLING.*

From the Departments of Pathology and Surgery, College of Physicians and Surgeons, Columbia University.

During the course of our studies on the effects of intestinal stasis on blood destruction and regeneration, some experiments were undertaken to determine the rôle of the liver and bile in the elimination of bacteria in normal fasting dogs. Several investigators[1] have found organisms in the tissues of apparently normal animals. Wolbach and Tadasu[2] found an anaerobic spore-bearing bacterium in the livers of 21 out of 23 healthy dogs. These authors obtained their material from dogs which were killed by chloroform. The interval between the last feeding and the death of the animal was not stated. They were unable to grow the organisms obtained from the liver in sterile bile, or bile containing media.

The material for our studies was obtained under strict aseptic conditions from 11 normal dogs which had been kept without food for 18 hours. Ether anesthesia was used. The specimens consisted of blood from the portal vein (obtained in 5 dogs), bile from the gall-bladder, and a wedge-shaped piece of liver measuring approximately 3 cm. x 2 cm. In addition, the organisms which were obtained from the liver tissue were incubated in undiluted and diluted bile (1:10 to 1:1,000,000). The organisms and bile were obtained from the same animal in each instance. Cooked meat dextrose broth was used as the culture medium. Strict anaerobiosis was maintained. Aerobes as well as anaerobes of intestinal origin develop satisfactorily under these conditions.[3] The liver tissue was also cultured aerobically.

In every dog the bile was sterile. The liver tissue from all the animals yielded a pure culture of an anaerobic spore-bearing bacterium which was similar to the one described by Wolbach. In 3 dogs the organism was identified as *B. welchii;* in the others the organism was not identified. It resembled the gram positive anaerobic bacillus commonly found in the intestine of normal dogs.

Cultures of the organisms in bile yielded the following results: in pure bile a sparse growth was obtained from 3 out of 11 dogs; in diluted bile, growth was obtained from all the dogs and in all dilutions (1:10 to 1:1,000,000).

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* This work was aided by a grant from the Eli Lilly Co.

In the 5 dogs from which the portal blood was cultured, no growth occurred.

The presence of living anaerobic bacilli in the livers of all our dogs, and their absence from the bile and portal blood, raises some interesting questions. The belief has been expressed that at times intestinal bacteria and their toxins get into the portal circulation, but if this is true, why should the above organisms survive in pure culture in the liver? Are we to assume that in the dog they alone can pass the intestinal barrier, or that the liver destroys all the others?

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[1] Reith, A. F., *J. Bact.*, 1926, xii, 367, with review of the literature.

[2] Wolbach, S. B., and Tadasu, S., *J. Med. Res.*, 1909, xxi, 267.

[3] Berg, B. N., Meleney, F. L., and Jobling, J. W., *Arch. Surgery* (in press).

3405

The Effect of Division and Transplantation of the Common Duct Upon Gall-Bladder Function.

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From the Departments of Pathology and Surgery, College of Physicians and Surgeons, Columbia University.

Recent studies[1, 2] tend to minimize the importance of the sphincter of Oddi in the regulation of gall-bladder activity. However, the methods which have been employed to eliminate the action of the sphincter are open to a number of criticisms. We have attempted to avoid them by completely dividing the common duct proximal to the sphincter and implanting the stump into another part of the duodenum. The effect of this procedure upon the action of the gall-bladder was determined by means of cholecystograms which were made according to the method described by Graham and his co-workers.[3]

Under ether anesthesia, the common duct in 2 dogs was isolated and doubly ligated just above its entrance into the duodenum. A linear incision 2 cm. long was made in the anterior surface of the duodenum about 8 cm. from the pyloric sphincter. The duct was divided ½ cm. proximal to the ligature, and fixed at the lower angle of the opening in the duodenum, which was then closed by two sutures.

Six weeks and 8 weeks respectively, after the operation, cholecys-

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* This work was aided by a grant from the Eli Lilly Co.

tograms were obtained in the following manner: 0.12 gms. sodium tetraiodophthalein per kilo body weight were injected intravenously. Eighteen hours after the injection of the dye, the first X-ray film was taken. Then the dogs were fed with the yolks of 3 eggs in 200 cc. of cream[4] and films were taken at intervals of 1, 3, 6 and 24 hours after the meal.

In both dogs, the shadow was normal in size and density at the 18th hour observation. Following the meal, in one dog, there was progressive shrinkage and increased density of the shadow, with complete disappearance at the 24 hour period. In the other dog, there was only a slight decrease in the size of the shadow, but there was a definite diminution in density, most marked after 6 hours; after 24 hours the shadow was still present. These findings are within the normal variations for dogs.

From the above it would appear that normal cholecystograms may be obtained in dogs after division and transplantation of the common duct. This excludes a reciprocal relationship between the sphincter of Oddi and the gall-bladder as promulgated by Meltzer.[5]

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[1] Burget, G. E., *Am. J. Physiol.*, 1926, lxxix, 130.

[2] Copher, G. H., and Kodama, S., *Arch. Int. Med.*, 1926, xxxviii, 647.

[3] Graham, E. A., Cole, W. H., and Copher, G. H., *J. Am. Med. Assn.*, 1925, lxxxiv, 1175.

[4] Boyden, E. A., *Anat. Rec.*, 1925, xxx, 333.

[5] Meltzer, S. J., *Am. J. Med. Soc.*, 1917, cliii, 469.

3406

Changes Observed in Epidermal Cells Covering Myxomatous Masses Induced by Virus Myxomatosum* (Sanarelli).

T. M. RIVERS. (Introduced by O. T. Avery.)

From the Hospital of the Rockefeller Institute for Medical Research.

Sanarelli,[1] in 1898, described an infectious myxoma indigenous to rabbits of South America. The characteristics of this disease have led investigators to classify it with the so-called filterable virus diseases. Tumor-like masses appear quickly at the site of inoculation, and later at various other points in the subcutaneous tissue, as well as in the lymph nodes and spleen. The disease is extremely malignant and usually kills rabbits within 7 to 15 days. Mammals and fowls that have been tested, with the exception of rabbits, have been found to be refractory to infection with the virus. No one has succeeded in cultivating the etiological agent on artificial media.

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* This virus became available for study through the kindness of Dr. C. E. Simon and Dr. A. Carrel.

The tumors, upon macroscopic and microscopic examination, present the picture observed in myxomas. At the same time, however, evidences of a severe inflammatory reaction are seen, *e. g.*, collections of polymorphonuclear leucocytes and other types of cells. Frequently the tumors do not appear unlike some of the myxomatous growths seen in chickens. Intracellular changes have also been observed and recorded. Splendore,[2] in 1909, stated that trachoma-like bodies occurred in affected cells. In 1911, Moses[3] reported that he was unable to demonstrate inclusions similar to the ones seen in trachoma. Aragao,[4] in 1911, described round bodies of uniform size in the hypertrophic nuclei of cells in the myxomatous masses. He thought that these bodies underwent division and represent the virus itself.

The point of particular interest concerning the myomatosis of rabbits and the occasion for the present paper is the fact that both epithelial tissue and connective tissue are affected. On the one hand, there seems to be a proliferation of certain cells within the connective tissue, while on the other, a destruction of epithelial cells is occurring. At the present time the changes observed in the epidermis will be described.

Rabbits were anesthetized at different times after infection, and small pieces of diseased skin were removed, fixed in Zenker's fluid plus 5 per cent acetic acid, sectioned, and stained with eosin and methylene blue.

Upon microscopic examination the first change noted in the epidermal cells is an increase in their size. Then small, pink, granular areas appear in the cytoplasm. These areas rapidly increase in size and frequently involve most of the cytoplasm. In the center of the acidophilic masses, blue, round or rod-shaped bodies are often seen. The cytoplasmic bodies in many respects resemble the Bollinger bodies found in cells affected by the virus of contagious epithelioma of fowls. The disease process in the epidermal cells progresses until there is a complete dissolution of the cells. At this time distinct vesicles appear in the epidermis, which are not unlike the ones encountered in virus diseases that usually attack the skin, *e. g.*, *herpes simplex*. The nature of the acidophilic masses in the epidermal cells described above is as poorly understood as is the nature of any of the inclusions that are associated with many other virus diseases.

Interest in the Rous chicken tumors has led to discussions in regard to their importance in the general study of malignant growths. The same questions propounded concerning the nature of the chicken tumors will undoubtedly be raised in regard to this rabbit myxoma. Although in this disease of rabbits there are myxomatous growths, the disease picture as a whole more closely resembles infectious processes than it does neoplastic ones. This difference is emphasized by the highly contagious nature of the rabbit disease, a condition never recorded in regard to chicken tumors. The fact that the pathological picture in the epidermis differs so much from the one occurring in the subcutaneous tissues makes the rabbit myxoma an extremely interesting disease. Immediately, one might ask whether more than one virus is being dealt with, inasmuch as it has been shown[5] that some tumors are excellent places for the growth and persistence of certain viruses. This question cannot be answered at the present time.

This is a preliminary report.

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[1] Sanarelli, G., *Centr. Bak., Abt.* 1, 1898, xxiii, 865.

[2] Splendore, A., *Centr. Bak., Abt.* 1, *Orig.*, 1909, xlviii, 300.

[3] Moses, A., *Mem. Inst. Oswaldo Cruz*, 1911, iii, 46.

[4] da Rocha-Lima, H., v. Prowazek, *Handbuch der pathogenen Protozoen*, 1920, Leipzig, ii, 959.

[5] Rivers, T. M., and Pearce, L., *J. Exp. Med.*, 1925, xlii, 523.

3407

Blood Groups Among the Yoruba Tribe of West African Negroes.

HENRY R. MULLER. (Introduced by J. H. Bauer.)

From the Laboratory of the West African Yellow Fever Commission of the International Health Board of the Rockefeller Foundation at Yaba (Lagos), Nigeria, W. A.

The Yoruba tribe of West African negroes, inhabiting the southwestern portion of Nigeria, W. A., is numerically large, consisting, it is estimated, of 2,000,000 individuals. Its members are distingguished by the tribal mark of three parallel horizontal scars on each cheek. Though this tribal marking has been strictly adhered to in the past, the younger generations are not marking their children in all instances, especially in the more cosmopolitan city of Lagos. The presence of these distinguishing marks was used by us to establish the individual's Yoruban descent.

During 1926 samples of blood from 325 individuals of the Yoruba tribe were collected in 0.9 per cent saline containing 2 per cent sodium citrate. After centrifuging and washing, suspensions of the red cells in normal saline were tested for agglutinable properties with known Group I and Group II human sera. These sera, free from preservative, were brought from New York in hermetically sealed 1.0 cc. ampules, and were kept continuously on ice up to the time of use. The open slide method was employed, in which 1 drop of serum was mixed with 1 drop of a heavy suspension of red cells on a slide. The reactions were rapid, clear cut, and easily readable with the naked eye, although the microscope was used from time to time as a check.

Adopting the Jansky classification, the results were:

Group I	—170	persons	52.3 per cent.
Group II	— 70	”	21.5 per cent,
Group III	— 75	”	23.0 per cent.
Group IV	— 10	”	3.2 per cent.

The bloods were obtained at the following cities:

Lagos	
Colonial Hospital, men	160
women	12
Prison: men	103
Abeokuta	
Baptist High School for Girls: women	15
Ibadan	
Prison: men	35

Thanks are hereby expressed to Dr. G. G. Butler, Pathologist at the Colonial Hospital at Lagos; to Dr. A. B. W. Smart, Medical Officer of the Prison at Lagos; and to Miss Olive Edens, acting superintendent of the Baptist High School for Girls at Abeokuta, all of whom aided in procuring the required samples of blood.

3408

Notes on the Growing of Seedlings for Physiological Experimentation.

RAYMOND PEARL and AGNES L. ALLEN.

From the Institute for Biological Research, The Johns Hopkins University.

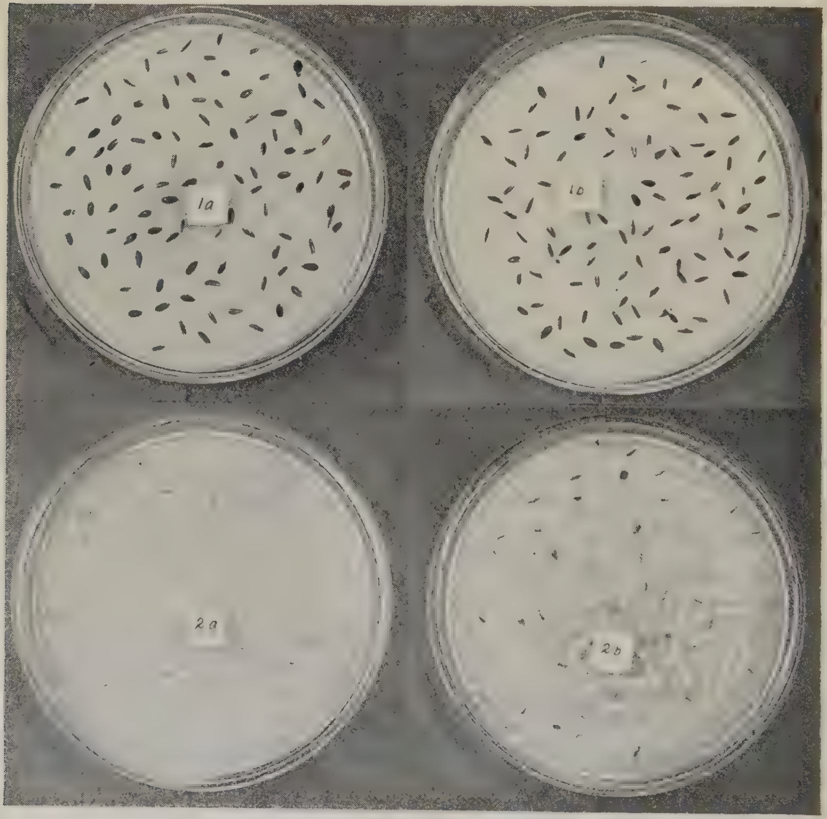
For some years past we have carried on rather extensive experimental work[1] with seedlings of the canteloup *(Cucumis melo, var.)*. Much effort has been devoted to the working out of a physically accurate, and at the same time biologically optimal technique. Considerable progress has been made towards the achievement of these desiderata, and it is the purpose of the present paper to report upon the main features of the standard basic technique which we are now using in our seedling work.

Most of our experiments are done under conditions of total darkness, with consequent complete etiolation of the seedlings. In the earlier stages of the work the seeds were germinated in the usual way between layers of moistened filter paper, cotton batting, or cloth, after preliminary soaking in water or some solution. They were then grown in water cultures in accordance with standard plant physiological technique.

All this procedure has now been abandoned in favor of a much better one. The seeds are germinated and grown upon a substratum of agar-agar. The best grade of bacteriological agar is bought at wholesale in bales of 100 or 200 lbs. in order to have a uniform supply over a long period of time. With the bale at present in use in this laboratory a concentration of 1.5 per cent gives the right consistency for the seedling work, making a firm, but not too firm jelly. For germination tests with this material, poured in Pyrex glass pie plates to a depth of about one inch, it is only necessary to strew the seeds to be tested on the surface of the agar jelly, and cover the dish with a glass plate to prevent evaporation. The 1.5 per cent agar is made with distilled water, and sterilized in the autoclave. With any given sample of commercial seeds we uniformly get a higher percentage of germination on these agar plates than with any standard germination technique, and we have tried them all. The use of agar in this way in seedling experimentation is not original with us. It has been employed by various persons, notably by Terroine,[2] who has used the method extensively in his studies on plant nutrition.

The most important advance which has been made in seedling technique in this laboratory has to do with the effect of removing the outer coat or testa of the seed before germination. This can be done mechanically, splitting the testa along its edges by compression in the plane of the margins of the seed. If carefully done, the embryo can be removed uninjured. The tegmen is usually not removed. From this point on the procedure is exactly as though one were dealing with the intact, entire seed. The shelled seed is soaked in water, sown on the agar plate for germination and growth, and in every way handled as an entire seed or seedling would be. For sterile culture work we have followed with about equal success either of two methods. The whole seed may be sterilized by immersion for 5 minutes in 1:1000 bichloride of mercury

FIG. 1.



Photographs of seedlings after 48 hours growth. 1a and b: Normal intact control seeds; 2a and b: Seeds from which testae had been removed from the start.

solution, and the testa then removed as an aseptic surgical procedure. This technique is based on the assumption that in the intact seed the embryo is sterile. Or the testa may be removed and the naked shelled embryo soaked for one minute in 1:1000 bichloride, and then rinsed off in sterile distilled water and sown on the sterile agar plate.

The advantages of removing the testa from the dry seed before starting any experimental procedure are: (1) higher percentage of germination, amounting almost invariably to 100 per cent of all fertilized seeds, (2) prompter germination, (3) much more rapid and vigorous growth of the seedling per unit of time. These facts are illustrated by the following typical experiment.

From a stock lot 400 seeds of *Cucumis melo* were taken, and divided in two groups (1 and 2) of 200 seeds each. The weights of the dry seeds in the two groups were as follows.

Lot 1 = 5.0473 grams.
Lot 2 = 5.0440 grams.

From each seed in Lot 2 the testa was removed in the way described above. Each lot was then divided into 5 batches of 40 seeds each, and each such batch was soaked for exactly 3 hours in 100 cc. of sterile distilled water. They were then removed, lightly blotted with filter paper, and sown on agar plates, 100 seeds to a plate, and put in a dark incubator operating at 30° C.

At the end of exactly 48 hours the plates presented the appearance shown in Fig. 1.

It is evident from the photographs that the shelled seeds had grown much better than the controls. After the photographs were made each seedling was carefully removed from the agar plate, and with a sharp scalpel the cotyledons were cut off from the sprout in each case. The fresh weights of the cotyledons and of the sprouts (stem + root) were then immediately determined. The material was then put in an oven operating at 98° C. and dried to constant weight. The results are shown in Table I.

From this table it appears that the 48-hour growth of the shelled seeds (stems + roots) was nearly 153 per cent in excess in fresh weight, and 87 per cent in excess in dry weight, over that of the normal control seeds.

This excess growth of the shelled seeds is probably mainly due to more rapid and greater water absorption by the cotyledons and to better oxygen supply. The testa is a protective envelope for the embryo, but apparently the plant pays a rather high physiological price for the protection, chiefly in the form of reduced permeability

TABLE I.

Growth of seedlings—Cucumis melo.

Seeds.	No. of germinated seeds	Per cent germination	Fresh weights. Grams.		Dry weights. Grams.	
			Cotyledons	(Stem + roots)	Cotyledons	(Stem + roots)
Lot 1. (Intact controls)	191	95.5	4.6234	2.4543	2.6040	0.2330
Lot 2. (Testa removed)	200	100	5.9591	6.2010	2.6166	0.4352
Excess of Lot 2 over Lot 1 Absolute	+9	—	+1.3357	+3.7467	+.0126	+0.2022
Excess of Lot 2 over Lot 1 Per cent	+4.7	—	+28.9	+152.7	+0.48	+86.8

even to so physiologically desirable a substance as water. Table I shows that whereas the dry weight of the cotyledons was practically identical in the two series, the cotyledons of the seeds in Group 2 absorbed nearly 29 per cent more water during the experiment than was able to get through the seed coats in Lot 1.

The greater immediate water absorbing power of the shelled seeds is demonstrated by the following typical experiment: From a stock of seeds of *Cucumis melo* two samples (A and B) of 100 seeds each were drawn. Lot A was the control. From each seed in Lot B the testa was removed. After weighing each lot was soaked for exactly 3 hours in 100 cc. of distilled water at room temperature. The seeds were then removed, lightly blotted with filter paper, and immediately weighed. The results are shown in Table II.

From Table II it is seen that the seeds in the B series absorb a higher percentage of water, in proportion to their initial dry weight.

TABLE II.
Water absorption by seeds. Cucumis melo.
(All weights in grams)

Seeds	Initial wgt. of dry seeds	Weight when put in H_2O	Weight after soaking 3 hrs.	Weight of water absorbed	Per cent wh. water absorbed is of weight when put in H_2O	Weight of cotyledons after soaking 3 hrs.
Lot A. (Intact control)	2.4920	2.4920	3.1597	0.6677	26.8	1.8064
Lot B. (Testa removed)	2.4918	1.5880	2.1068	0.5188	32.7	2.1068

If we suppose, as seems entirely justifiable, that the initial dry weight of the *cotyledons* alone was approximately the same in the two series, it then follows from the last column in Table II that at the end of the period of soaking the cotyledons of the intact seeds of Lot A had absorbed 0.2184 gr. of water, or 13.75 per cent of their assumed initial weight, while the shelled cotyledons of Lot B had absorbed 0.5188 gr., or 32.7 per cent of their initial weight. This is a complete paper.

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3409

Ocular Manifestations in Anaphylaxis.*

BRET RATNER, HOLMES C. JACKSON, AND HELEN LEE GRUEHL.

From the Department of Physiology, University and Bellevue Hospital Medical College, New York University.

In our experiments with guinea pigs, in which anaphylactic symptoms were induced either by subjecting sensitized animals to a dust-laden atmosphere or by parenteral injection, we have noted that there also occurs, in some cases, a suffusion of one or both eyes.

This suffusion at first is thin and watery, rapidly becoming thick and creamy, and in some cases eventuates in the eyelids becoming matted together. This suffusion lasts for half an hour and sometimes longer.

These symptoms were manifested more frequently in sensitized animals exposed to dust than in those animals injected parenterally. These manifestations were not observed in animals which died rapidly in anaphylactic shock, accounting for the greater percentage of positive results in those exposed to the dust.

Out of a total of 523 positive anaphylactic experiments, 53, or 10.1 per cent, showed these ocular symptoms. Symptoms were noted in 15.3 per cent of the dust inhalation experiments, 8.3 per cent of the experiments with intravenous injection, and 4.5 per cent of the experiments with intraperitoneal injection. (See table.)

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* This work is being carried on under "The Crane Fund for the Study of Anaphylaxis."

TABLE I.
Ocular Symptoms in Positive Anaphylaxis.

Positive Anaphylactic Exp.		Ocular Symptoms			Per cent Ocular Symptoms
Method	Number	One eye	Both eyes	Total	
Cage*	157	6	18	24	15.3
Intravenous	322	3	24	27	8.3
Intraperitoneal	44	—	2	2	4.5
Total	523	9	44	53	10.1

* Cage indicates exposure to dry dander dust.

These figures indicate that this symptom is of comparatively infrequent occurrence.

An animal showing this eye symptom and recovering after one anaphylactic response may manifest a similar eye reaction during a second or third anaphylactic reaction.

Further, an animal manifesting this eye symptom after dust contact may subsequently manifest it after an intravenous injection. An interesting example of this was the case of only one animal in which a suffusion of the right eye occurred upon contact with horse dander dust, and when subsequently injected intravenously with horse dander solution, it again showed suffusion in the same eye.

This symptom must undoubtedly be of an anaphylactic nature and not a local irritative one, because we obtained the reaction from exposure to the dust and after intravenous injection of dander solution.

Vernal conjunctivitis, an eye condition usually occurring in children, and possibly some other forms of conjunctivitis of unknown etiology, may have some relationship to anaphylaxis. In a recent publication by Lehrfeld[1] a series of cases of vernal conjunctivitis was reported with positive allergic skin reactions to pollens, feathers, etc. This investigator suggests that this disease is of an allergic character.

There may be some relationship between the symptoms we have described in the guinea pig and such conditions observed in the human being.

This is a complete paper.

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3410

The Antirachitic Effect of December Sunlight: Seasonal Variation.

FREDERICK F. TISDALL AND ALAN BROWN.

From the Nutritional Research Laboratories of the Sub-Department of Paediatrics, University of Toronto, and of the Hospital for Sick Children, Toronto.

It was first shown by Huldschinsky[1] that rays from the mercury vapor quartz lamp produced a definite antirachitic effect on rachitic infants. Hess and Unger[2] demonstrated that the sun's rays also prevented infantile rickets. This beneficial effect of the sun was confirmed with carefully controlled animal experiments by Powers, Park, Shipley, McCollum and Simmonds.[3] Hess and Weinstock[4] found that the rays which exerted this curative effect were shorter than 313 millimicrons. This means that the effective solar rays range only from 313 to 290 millimicrons in length, and constitute a very small percentage of the total solar radiation. As these very short rays are readily absorbed by smoke, dust and moisture in the atmosphere, the ultraviolet content of the sun's rays is markedly reduced in the winter months, when the sun is low in the sky, and the rays consequently have to pass through a greater distance of our atmosphere.[5, 6] It is, therefore, reasonable to expect that the antirachitic effect of sunlight will be correspondingly reduced. This raises the question as to whether this reduction may not be so great as to practically eliminate any beneficial effect during the winter months.

The results here reported are some of those obtained in an experiment being conducted by this Department with the cooperation of the Department of Physics, and the Department of Physiological Hygiene of the University of Toronto, in an attempt to determine the variations in the ultraviolet content of the antirachitic effect of the sun's rays throughout the year. The experiment was commenced in September, 1926. Each week a series of young rats are placed on a rickets-producing diet and exposed daily to the direct rays of the sun. The rats are killed after the desired period, roentgenograms of the bones made, the inorganic phosphorus content of the whole blood estimated, and the percentage of ash in the bone determined. Daily measurements of the ultraviolet content of sunlight are also made. Some of the results obtained during the month of December with the animal portion of the experiment are of sufficient interest to justify publication now.

The rats used were albinos, all obtained from the same stock.

TABLE I.

Effect on rats of exposure to December sunlight.

Treatment	Inorganic Blood Phosphorus mg. per 100 cc.			Per cent ash in bone		Roentgen Ray Evidence of Rickets	
Rats started Nov. 29, 1926. Initial Blood P. = 7.5 mg. per 100 cc. Initial ash in bone, 40.0 per cent.	After 1 wk.	After 3 wks.	After 4 wks.	After 3 wks.	After 4 wks.	After 3 wks.	After 4 wks.
Normal diet—inside cage	—	—	7.0	—	—	—	none
Rachitic diet—inside cage	—	—	1.5	—	—	—	marked
Rachitic diet—outside ¼ hour daily	—	—	1.8	—	—	—	marked
Rachitic diet—outside 2 hours daily	—	—	2.6	—	—	—	slight
Rats started Dec. 6, 1926. Initial Blood P. = 7.3 mg. per 100 cc. Initial ash in bone, 40.0 per cent.							
Normal diet—outside 2 hours daily	5.6	7.1	6.5	52.8	56.7	none	none
Rachitic diet—inside cage	3.0	3.0	1.2	31.8	30.3	marked	marked
Rachitic diet—outside 1 hour daily	—	3.0	1.7	34.0	35.9	moderate	marked
Rachitic diet—outside 2 hours daily	5.0	3.9	2.9	39.6	43.7	moderate	slight to moderate

They were 25 to 27 days old and weighed about 40 grams. They were placed on McCollum's diet 3143 (wheat 33 per cent, corn 33 per cent, wheat gluten 15 per cent, gelatine 15 per cent, calcium chloride 3 per cent and sodium chloride 1 per cent). The rats exposed to sunlight were placed on the roof of the hospital at 11 o'clock each day and taken in, after exposure varying from 15 minutes to 2 hours. Although the rats were exposed up to 2 hours daily they did not receive 2 hours of sunshine each day. The records of the Dominion Meteorological Department, which is situated within half a mile of the hospital, show that the average daily sunshine from 11 to 1 o'clock during the month of December was only 0.6 of an hour (36 minutes). Control rats were kept inside in an ordinary well lighted room on the same diet. Further control rats were placed on a normal diet, some being exposed to sunlight 2 hours daily, and others being kept inside. After varying periods up to 4 weeks, 2 rats from each cage were killed by bleeding from the carotid artery, under light ether anesthesia. Roentgenograms were taken of the rats.* The inorganic phosphate content of the blood was estimated by the method of Tisdall,[7] and the ash content of the bone by the procedure outlined by Betke, Steenbock and Nelson.[8]

The inorganic whole blood phosphorus and the per cent ash in the bones are recorded in Table I. In the same table is also given the degree of rickets as observed from the roentgenograms. Although the roentgenograms of the rats exposed to the available sunlight for a period of ¼ to 1 hour showed marked rickets, a definite deposition of lime salts could be seen at the ends of the shafts of the long bones, which deposition was not present in the rats kept inside in the same diet.

Conclusion: The sun's rays in December in the latitude of the City of Toronto produce a definite antirachitic effect on rats fed on a rickets producing diet.

Our thanks are due to the Department of Health of the Province of Ontario for a grant in aid of the expense of this investigation and to the Honorable Dr. Forbes Godfrey, Minister of Health, for his interest in this work and the authorization of the grant from his department. This is a complete report.

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* The authors are indebted to Dr. A. H. Rolph of the X-ray Department of the Hospital for Sick Children for the roentgenograms.

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3411

The Antirachitic Effect of December Skylight and of December Sunlight Through Vitaglass.

FREDERICK F. TISDALL AND ALAN BROWN.

From the Nutritional Research Laboratories of the Sub-Department of Paediatrics, University of Toronto and of the Hospital for Sick Children, Toronto.

In the preceding publication,[1] results were reported which indicate that December sunlight in the latitude of the City of Toronto possesses a definite antirachitic effect. Although the rats were exposed for 2 hours daily, they received only a daily average of 36 minutes of sunshine, due to the cloudy weather. It has been noted by Dorno[2] and Hill[3] that skylight contains ultraviolet rays, as well as direct sunlight. This raises the question as to how much of our antirachitic effect was due to the 36 minutes exposure to sunshine, and how much to the remaining 84 minutes exposure to skylight. Accordingly rats on McCollum's rickets producing diet No. 3143[1] were placed outdoors for 2 hours daily from 11 a. m. to 1 p. m., exposed to skylight, but protected from direct sunlight. After varying periods ranging from 1 week to 4 weeks, 2 rats at a time were killed, roentgenograms taken of the bones, the blood phosphorus determined, and the per cent ash in the bones estimated. The results which are given in Table I, indicate that December skylight possesses a definite antirachitic effect, which effect is almost as marked as that obtained by exposure to the available sunlight.

A clear glass which the manufacturer claims will transmit the shortest ultraviolet rays of sunlight (290 millimicrons) can now be obtained under the trade name of "Vitaglass". We found that rays as short as 262 millimicrons, the source being an iron arc in air, will pass through this glass. To get some idea of its value from the clinical standpoint, we constructed from vitaglass, a glass box to fit over and around one of the cages. Another glass box made with ordinary glass which transmitted rays only as short as 320 millimi-

TABLE I.

Effect on rats of exposure to December skylight; effect of December sunlight through vita glass.

Treatment	Inorganic Blood Phosphorus mg. per 100 cc.			Per cent ash in bone		Roentgen Ray Evidence of Rickets	
Rats started Dec. 6, 1926. Initial Blood P. = 7.5 mg. per 100 cc. Initial ash in bone, 40.0 per cent.	After 1 wk.	After 3 wks.	After 4 wks.	After 3 wks.	After 4 wks.	After 3 wks.	After 4 wks.
Normal diet—outside 2 hours daily	5.6	7.1	6.5	52.8	56.7	none	none
Rachitic diet—inside cage	3.0	3.0	1.2	31.8	30.3	marked	marked
Rachitic diet—sunlight 1 hour daily	—	3.0	1.7	34.0	35.9	moderate	marked
Rachitic diet—sunlight 2 hours daily	5.0	3.9	2.9	39.6	43.7	moderate	slight to moderate
Rachitic diet—skylight 2 hours daily	4.6	3.4	2.4	41.4	40.5	moderate	moderate
Rats started Nov. 22, 1926. Initial Blood P. = 7.5 mg. per 100 cc. Initial ash in bone, 40.0 per cent.							
Normal diet—outside 2 hours daily	—	—	5.5	—	61.4	—	none
Rachitic diet—inside cage	—	1.6	—	—	—	—	marked
Rachitic diet—outside ½ hour daily	—	2.5	1.4	—	38.1	—	marked slight healing
Rachitic diet—exposed 2 hours daily to sunlight through vita glass	—	2.4	1.6	—	31.2	—	marked slight healing
Rachitic diet—exposed 2 hours daily to sunlight through ordinary glass	—	1.6	1.6	—	28.5	—	marked

crons, was also made. Rats on a rickets-producing diet were then exposed to sunlight from 11 a. m. to 1 p. m. daily, the rays in one case passing through vitaglass and in the other through ordinary glass. The rats were then killed and examined. The results which are recorded in Table I indicate that the rays passing through the ordinary glass have no antirachitic effect, while the rays passing through vitaglass have roughly one-quarter the value of the direct rays.

The cold air is apparently not a factor in producing any of the changes which we have attributed to the ultraviolet rays, as the cold air circulated freely into the cage enclosed with ordinary glass with no resultant prevention of rickets.

Conclusions: (1) December skylight in the latitude of the City of Toronto produces a definite antirachitic effect on rats fed on a rickets producing diet.

(2) The rays from December sunlight which have passed through vitaglass had an antirachitic effect which is roughly one-fourth the value of the direct rays. Rays which have passed through ordinary glass had no antirachitic effect.

Our thanks are due to the Department of Health of the Province of Ontario for a grant in aid of the expense of this investigation and to the Honorable Dr. Forbes Goodfrey, Minister of Health for his interest in this work and the authorization of the grant from his department. This is a complete report.

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[2] Dorno, quoted by Hill, L., *Brit. J. Rad.*, 1926, xxxi, 25.

[3] Hill, L., *Brit. J. Rad.*, 1926, xxxi, 25.

3412

A Chemical Study of the Normal Aqueous Humor.*

A. M. YUDKIN, A. C. KRAUSE, H. H. GOLDSTEIN AND M. B. BERMAN.
(Introduced by S. C. Harvey.)

From the Ophthalmological Section of the Surgical Department, Yale University School of Medicine, New Haven, Conn.

The preliminary study of the composition of the normal aqueous humor in the dog was undertaken to serve as a basis for further investigation of the changes which occur as a result of chemical and physical alterations in the eye. The determination of total nitrogen,[1] non-protein nitrogen,[2] protein nitrogen, amino-acid nitrogen,[3] urea nitrogen,[3] uric acid,[4] creatinine,[2] glucose,[5] and refractive index was made. Because of the small quantity of eye fluid, the ordinary methods for the estimation of these substances were modified by the employment of smaller quantities of reagents, and by the use of a microcolorimeter. The refractive index was obtained by the means of a Pulfrich-Zeiss dipping refractometer. The fluid was removed by paracentesis of the anterior chamber, with a fine bore needle, under ether, amytal or cocaine anesthesia as indicated in the table. About 0.6 to 0.8 cc. of aqueous humor was obtained each time. It was found that 0.2 cc. was sufficient for any one of the analyses except that for amino-acid nitrogen, for which 0.5 cc. was required.

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* The expense of this experimentation was defrayed in part by a grant from the Committee on Scientific Research of the American Medical Association.

The following table shows the refractive index and the average results in milligrams per 100 cc. of eye fluid. This is a preliminary report.

TABLE I.

	Anasthesia: Cocaine locally. No. of dogs used.	Average mg. per 100 cc. of aqueous humor.	Anesthesia: Ether. No. of dogs used.	Average mg. per 100 cc. of aqueous humor.	Anesthesia: Amytal. No. of dogs used.	Average mg. per 100 cc. of aqueous humor.
Total nitrogen	18	40	3	35	6	41
Non-protein nitrogen	23	25	3	21	6	32
Protein nitrogen	14	14	3	13	6	9
Amino-acid nitrogen	14	9	—	—	—	—
Urea nitrogen	15	12	—	—	—	—
Uric acid	23	1.8	9	2.0	9	1.9
Creatinine	23	1.3	5	1.4	6	1.2
Glucose	36	77	17	137	6	83
Refractive index	39	1.33505	17	1.33528	6	1.33506

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3413

The Histogenesis of Renal Casts.

HENRY JACKSON, JR.

From the Thorndike Memorial Laboratory, Boston City Hospital, and Department of Medicine, Harvard Medical School.

During the study of some artificially nephropathic animals, the probable origin of the so-called circular reticulum of the kidney, and of the well known urinary casts, has been found.

Under the influence of a pathological environment the cells lining the convoluted tubules swell toward their tip and become vacuolated. The swelling continues until there is found a globular bulb, containing some of the cytoplasmic granules, and surrounded by a thin membrane. This bulb is gradually pinched off from the parent cell and comes to lie, with its neighbors, free in the lumen of the convoluted tubule. Such a collection of cell extrusions constitutes the circular reticulum. Each unit is globular in shape and contains more or less cytoplasmic granules. The limiting membrane of these globular bodies dissolves next and the granules contained within become massed together. At first the granules are large and stain deeply with phosphotungstic acid. At this stage they occur in clumps separated by circular clear areas. The granules gradually become finer, stain more intensely with eosin, and less intensely with phosphotungstic acid, and are changed into a more or less homogeneous mass, whose surface is granular. As this mass descends through the collecting tubules, the surface becomes smooth, or nearly so, and it emerges at last as the familiar cast.

The first stages, globe-like bulbs, are found only in the convoluted tubules. Various modifications of the granules occur in the loops of Henle while the true cast and its immediate predecessor are found only in the collecting tubules.

Exactly the same process has been followed in sections of human kidneys, though the first stages are difficult to find owing to the ease with which they disintegrate after death. This is a preliminary report.

3414

The Narrow-Path Elevated Maze for Studying Rats.

W. R. MILES.

From the Psychology Laboratories, Stanford University.

Students of animal behavior concern themselves much with the problem of "learning," and the influence that various factors may have upon it. The devices commonly employed in working this field are problem boxes and mazes.

A very unique maze was employed by Vinçent[1] in a study on the use made of the vibrissae by the rat. The runways in this case were raised 2 feet above the laboratory floor, the paths were 4 inches wide and the walls which should have enclosed the runways were hinged at the lower edges so that they dropped below. This system of raised paths (not alleys) covered an area 54x54 inches and the open space separating adjoining parallel runways was 6 inches. The food box except where it connected with the maze was surrounded with 4 inch walls. Vincent reports that fewer errors were made on the "sideless maze" than when it was converted into an alley maze by turning up the hinged sides and hooking them together. The use of nose, vibrissae, feet and apparently eyes was easily noted with this elevated maze, but Vincent's study probably has not been widely or carefully read, otherwise many would have been prompted to try this type of device.

The narrow-path elevated maze illustrated in Fig. 1 differs in several respects from the Vincent "sideless maze". It is an assembly of straight units which are stood up adjoining each other, and hence may be placed in many different combinations. There are 19 of these units in the pattern illustrated. The rat is started at S and in the illustration (see below the arrow at F) is shown eating at the "food-box". This latter is an inverted bottle cap fastened in a notch at one end of a maze unit so that the food is not observable above the surface of the paths. A similar food cup is shown on the isolated support 0 in the figure.

Each hurdle-like frame (made of redwood) provides a horizontal path 1 inch wide and 36 inches long. The horizontal strip, ¾ inch thick, is supported 30 inches, table height, above the floor by two uprights suitably braced together. Each upright has a 4x6 inch base block. At each end the horizontal bar projects 3 inches beyond the support. These units lend themselves particularly to T-type patterns. The one shown employs 19 maze frames, presents the animal with 14 T-choices and 1 L-choice, 15 culs-de-sac each 18 inches long and a true path 32 feet in length. The open space between adjoining parallel paths is 18 inches. Most of the runways between turns are 18 inches, but some are 3 feet and two are 4½ feet long.

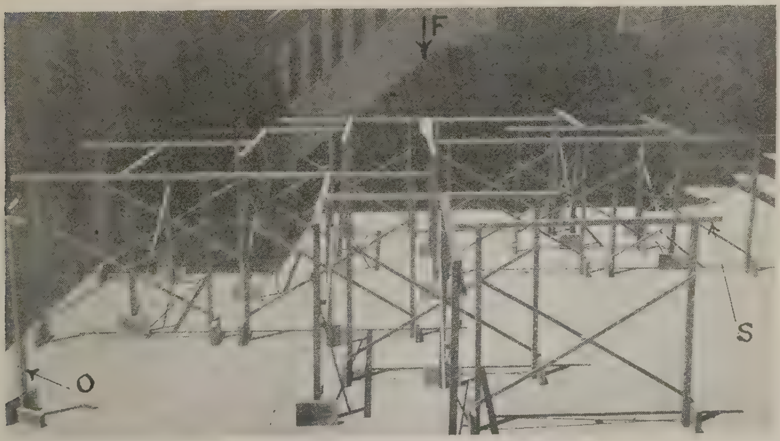
Such a set of narrow elevated paths makes what might be termed a *skeleton maze,* the form of which is readily modifiable. Cross-sectional area has been reduced to the least practical minimum, the situation is simplified for the animal, he is free to use vision along with other sensory cues, and every move may be readily observed. Such a maze stacks compactly when not in use. The parts, with the exception of the frame that holds the tiny food cup, are differently placed each time, thus pure pattern is emphasized. Excrements are not so bothersome a factor as in alley mazes.

The research in progress with this maze will be reported later. At present some general points concerning the behavior of the rats may be mentioned.

1. A 1-inch elevated path is wide enough for large adult rats to run with ease at least after the first trial or so. They can turn around without special difficulty at the end of a "jump-off" as elsewhere. Two 400 gram males can even pass each other; one burrows under. Younger rats could doubtless use a slightly narrower width, but the 1 inch path is practical.

2. From several months experience accidental falls are found to be very rare,* and the animals practically never attempt to climb down. The sections are not tipped over. The slight vibration of a frame may cause an animal to stop momentarily.

FIG. 1.



Elevated narrow-path, maze for rats.

3. After preliminary feedings on two adjoined units animals commonly explore rapidly. Quickly exhausting the area at any one location they go on. Rats well used to this kind of maze run the pattern in Figure 1 without mistake on as early as the 4th to 6th trial, and some well used to the pattern have covered it in as short as 10 sec. Corners may be cut.

4. Tracking does not seem to play a very important rôle. Each maze unit may be quickly turned end for end. All the culs-de-sac will thus be placed in the true path. This seldom seems to make any difference in the next run.

5. Vision is often used in general orientation as may be shown by the promptness with which many animals notice a maze unit that is set in somewhere out of contact with the general pattern. They will reach and often cross the 5 inch gap to this isolated unit (which may be new or old), explore it and then return.

6. Balance at rapid turns or at the time of a mis-step is obviously aided by movements of the tail. Not infrequently the animal may stand high up on its hind legs. This was noted by Vincent.

7. The introduction of short cuts, alternate paths, gaps and other obstructions produce easily observable changes in behavior, which may be recorded by motion pictures apparently without distraction to the rat. This is a preliminary report.

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[1] Vincent, S. B., *Behavior Mono.*, 1912, i, 1-81.

* Vincent reported that falls happened from the ends, turns and ''frequently from sides midway.'' Our experience does not agree. Possibly her animals were too well used to the same floor pattern in the alley form, *i. e.*, with the sides turned up.

3415

Observations On Hearing-Acuity For Bone-Transmitted Sound.

A. G. POHLMAN and F. W. KRANZ.

From the Department of Anatomy, St. Louis University, St. Louis, Missouri, and the Riverbank Laboratories, Geneva, Illinois.

The writers have demonstrated that bone-transmitted sound follows at least part of the pathway to the labyrinth taken by air-transmitted sound. It has also been determined that the acuity for air-transmitted sound is not necessarily a criterion for the bone-sensitivity, or *vice versa*. The writers have reported elsewhere[1] the quantitative findings in two cases of deafened individuals who claim to hear better in a noise. While these two individuals belong to the *paracousis Willisi* type, they were found not to have unusual bone-sensitivity. Deafened people do not really hear better in a noisy place; rather it is the normal individuals who do not hear so well.

Acuity for Air-Transmitted Sounds. Figures 1 and 2 show the acuity for air-transmitted sound of the two subjects included in this report. The acuity is given in terms of the logarithm of the reciprocal of the necessary intensity for hearing, and a difference of one unit in sensitivity corresponds to an intensity ratio of 10, two units

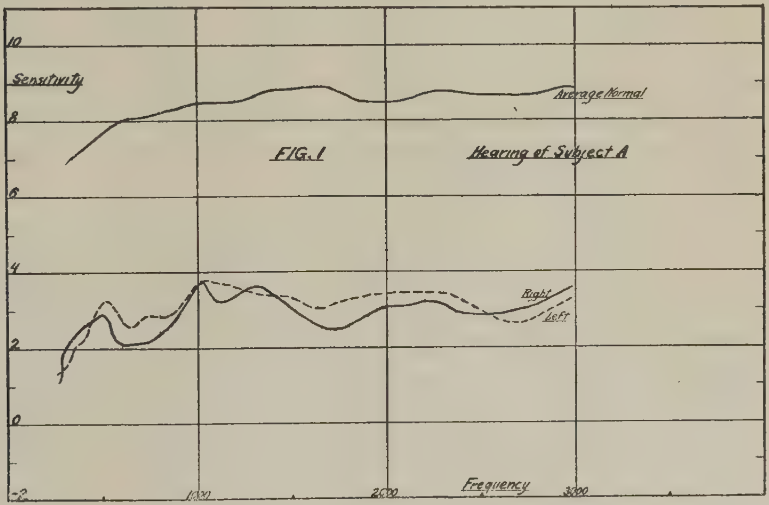

FIG. 1

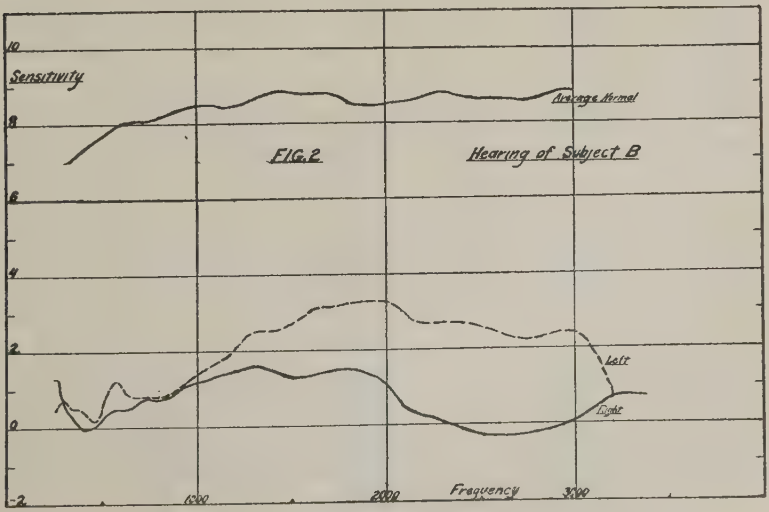

FIG. 2

to 100, etc. The average sensitivity of normal hearing people is also shown.

The sensitivities of the two ears of subject A are about alike and average about 3.0, while the average normal curve has a height of about 8.5, giving a difference of 5.5. This would indicate that it takes about $10^{5.5}$, or about 300,000 times normal intensity for subject A to hear. Similarly subject B requires about 100,000,000 times normal intensity for the right ear, and 3,000,000 times normal intensity for the left ear. Qualitatively, subject A by paying attention, experiences no marked difficulty in conversing with normal people. On the other hand, normal people must speak very loudly in order to make subject B understand them. Incidentally it may be remarked that subject A hears conversation only a trifle better with ears open than with external auditory canals occluded.

The measurements were taken with the continuous-tone-range audiometer previously described,[2] and a calibrated telephone receiver was used for the sound production.

Acuity for Bone-Transmitted Sounds. Figure 3 shows the acuity of the two subjects for vibrations transmitted to the forehead by means of the bone-activating receiver, as described in previous papers.[3] The average normal level for bone-transmission acuity was obtained by averaging the measurements on several observers of normal air-acuity. The figures at the left show the comparative in-

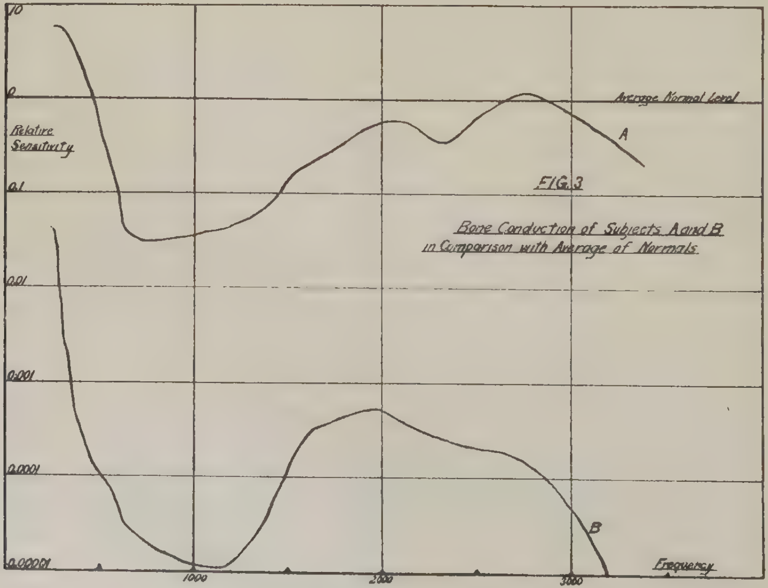

FIG. 3

Bone Conduction of Subjects A and B in Comparison with Average of Normals tensities of vibration. The form of the curves for the two subjects is similar. The bone-acuity curve of A shows a departure from the average normal about equal to the most extreme departures in the 7 individual measurements used in obtaining this norm. The curve for subject B shows very distinct deficiency in bone-acuity. This deficiency amount to as much as a factor of 100,000 times normal for part of the frequency range. In the region of 1,000 cycles per second and 3200 cycles per second 100,000 times the normal amount of energy applied through the bone-activating receiver was required to produce an audible impression upon subject B.

The effect on bone-sensitivity through occlusion of the external auditory canals with dental impression compound was tried and found to be negligible in both subjects. The small differences were well within the observational error. This observation differs from the results obtained on normal-hearing people given in a previous paper. The occlusion of the external auditory canals improved the average bone-acuity in normal-hearing individuals. This improvement, tested from the forehead, increased with the decreasing frequency from 2600 cycles per second to 256 cycles per second, and amounted to a factor of about 50 in the required energy at 256 cycles.

Acuity for Hearing Under Other Test Conditions. On testing subject A with tuning forks of 256 and 512 cycles per second, it was found that he could hear these forks placed at his open mouth as well as he could when placed at his external auditory canals. He did not hear these forks when placed at the side of his cheek. Clenching the teeth also appeared to make a difference in his acuity for air-transmitted sound. Tests were therefore made on subject A for various conditions of hearing air-transmitted sound at frequencies from 256 to 1500 cycles per second. The sound was produced by a telephone receiver lying on a soft pad about 30 inches in front of the observer. The acuity under the different conditions imposed was compared by varying the current through the receiver and measuring the minimum current necessary for hearing the tone under each condition. The observations were taken in a room lined with felt and interference effects from standing waves were at a minimum.

The different conditions tested were: 1. with mouth closed; 2. with mouth closed and teeth clenched; 3. with mouth wide open; 4. with mouth open to form an aperture and cavity such as to tune in or be resonant with the tone presented, as if whistling; and 5. an acoustical fan held between the teeth.

The acoustical fan is made of flexible hard rubber, fitted with a string which serves to pull the surface of the fan into a bowed or bent form and maintain it in this position. The fan is held by the handle with the top of the fan bent toward the person and the upper edge held firmly between the teeth.

TABLE I.

Acuity of Subject A under various conditions.

Frequency	Mouth Closed		Mouth Open		
	Mouth closed	Teeth clenched	Wide open	Mouth tuned	Fan used
384	Nothing heard	1.	1.3	67.	440.
512	1.	4.	1.3	29.	76,000,
768	1.	1.	1.	670.	12,100.
1024	1.	1.	0.4	—	2.1
1500	1.	1.	1.	—	1.

Table I gives the results of this series of tests. The numbers indicate proportional sensitivity under the various conditions. This proportionality, however, holds only for the different conditions at any one frequency, and not for the change of sensitivity with the change in frequency. Results on the frequencies of 256 and 320 cycles are not given in this table because the acuity was too low to obtain numerical results. However, for these two frequencies the conditions of clenched teeth and open mouth showed greater than normal sensitivity, and the fan was of distinct benefit.

When the fan was held between the teeth, the sensitivity was increased from 256 to 1024 cycles per second. Compared with the condition of closed mouth, the benefit derived was as high as a factor of 76,000 at 512 cycles per second. The advantage at 1024 cycles was too small to be certain or significant.

The only previously recorded measurements on the acoustical fan are given by Sabine.[4] In the individual tested, he found that there was perhaps a slight benefit at 256 and 512 cycles. The enhancement was not more than an intensity factor of 2, which is of negligible importance. He also found that this individual derived no benefit through use of the fan for conversational purposes. (This individual was diagnosed as a case of stapedial fixation.)

Clenching of the teeth was of advantage to subject A at the lower frequencies, but made no difference above 512 cycles. Opening the mouth was a slight aid to hearing at the lower frequencies. Tuning the mouth cavity in resonance with the pitch presented was of considerable help in acuity for the frequencies of 768 cycles and lower.

Subject B experienced considerable practical benefit to conversation through the use of the acoustic fan, in spite of the fact that his bone-acuity was so low that quantitative measurements were not possible. This subject derived no apparent benefit from opening his mouth, or attempting to tune his mouth cavity to the pitch to be heard, except at 1024 cycles, where the benefit was slight.

These tests with an acoustic fan would seem to indicate an available method which may be used by the otologist in determining the acuity for bone-transmitted speech; and if the diagnosis in the case reported by Sabine is correct, then the fan may be employed as an aid to the diagnosis of stapedial fixation. This is a complete report.

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[1] Pohlman, A. G., and Kranz, F. W., *Arch. of Otolarygol.*, 1926, iii, 136.

[2] Kranz, F. W., *Phys. Rev.*, 1923, xxii, 66.

[3] Pohlman, A. G., and Kranz, F. W., *Ann. of Otol., Rhinol. and Laryngol.*, 1925, xxxiv, 1224.

[4] Sabine, P. E., *Laryngoscope*, 1921, xxxi, 819.

3416

The Development of Marked Activity in Ergosterol Following Ultra-Violet Irradiation.

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From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Chemical Laboratory, University of Goettingen.

In a previous communication we showed that cholesterol which has been purified by means of bromination failed to develop antirachitic properties as the result of ultra-violet irradiation.[1] These experiments, as well as some others, led us to question whether it is the cholesterol itself which becomes active or some contaminating substance intimately associated with it.

Recently we have been conducting experiments with a preparation of ergosterol prepared from yeast. Ergosterol is an optically active sterol possessing three double bends and a hydroxyl radical. Its molecule therefore possesses two factors which have been found to be linked with the activation of cholesterol derivatives and allied sterols.[2] The ergosterol was irradiated with the mercury vapor lamp for one-half hour at a distance of one foot, then suspended in linseed oil and fed to rats in varying amounts. It was found to bring about a healing process of the bones when even as little as 0.003 mg. per capita daily was given. In tests in which irradiated cholesterol is fed, it has been found that approximately 1 mg. is needed to initiate healing.

Other experiments will be undertaken to ascertain the relationship of ergosterol to cholesterol and the extent of its distribution in the animal body. This is a complete report.

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[1] Hess, A. F., and Windaus, A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 171.

[2] Hess, A. F., and Windaus, A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 369.

3417

Proteins and Non-protein Colloids as Bioelectric Models.

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From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville.

The following experiments were undertaken in order to test the rôle of colloids in the production of electric currents in tissues. Hober[1] and his collaborators, Matsuo,[2] Mond,[3] and Deutsch[4] have tested cell arrangements with various proteins as middle conductors, *e. g.,* 1/10 mol. solution of KCl gelatin, 1/10 mol. solution of LiCl + about 0.01 volt, and compared these with other arrangements in which various animal or vegetable tissue took the place of gelatin.

Generally an agreement has been found. It has not yet been shown, however, whether non-protein colloids like agar agar might not act in the same way as proteins. To ascertain this, analogous cells with agar agar, etc., were measured and compared with the protein cells. With protein cells the following electromotive forces have been observed, m/10 KCl being constantly on the one side of the proteins, 1/10 mol. solutions of various solutions on the other side.*

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* The technique of these measurements is simple. The protein is poured into a glass tube, open at both ends, filling the entire lower half of the tube as a plug. The upper end of the plug is constantly in contact with m/10 KCl, and this again with a saturated KClHgCl electrode; the lower end of the plug is dipped successively into the various salt solutions, which in turn are in contact with the second electrode. A binant electrometer was used for measuring the electromotive forces in the present research, but a compensation apparatus with a galvanometer would do the same service. Control tests in which the lower end of the protein plug dips also into m/10 KCl are always necessary. This symmetrical arrangement should show no electromotive force, but it usually does, consequently this e. m. f. must be subtracted from all the value observed.

TABLE I.

Electromotive forces in milli volts with the following proteins as middle conductors.

Variable salt solution (against KCl)	Neutral gelatin tannate observed by			10% gelatin observed by Writers	Euglobulin (Mond)	Casein (Mond)
	Matsuo	Mond	Writers			
	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts
NaCl	1	7	9	7	25	15
LiCl	4	12	15	12	27	21
$(C_3H_7)\ NH_3Cl$	4.5		19	9		
$(CH_3)\ NCl$	6		19	13		
K_2SO_4	—7	—16†	—15	—13	—28†	—22†
Na salicylate	2.5		—6	—10		

† These measurements of Mond are made with 1/10 NaCl against 1/10 Na_2SO_4. One can assume that this approximately equals the value of 1/10 KCl against 1/10 K_2SO_4.

This shows that the result of the measurements varies within wide limits. Even for the same protein (gelatin tannate) Matsuo and Mond, both working under Hober's direction, obtained quite different figures. The range of the electromotive forces observed for all the proteins cited, is given in the following table, and it is to be compared with the electromotive forces observed on agar agar, starch and kaoline.

TABLE II.

	Range of e.m.f. on various proteins	e. m. f. on agar agar	e. m. f. on starch paste	e. m. f. on kaoline
	millivolts	millivolts	millivolts	millivolts
For NaCl	1 to 25	7	8	7
For LiCl	4 to 27	10	18	11
For $(C_3Hg)\ NH_3Cl$	4 to 19	12	13	10
For $(CH_3)_4\ NCl$	6 to 19	13	13	10
For K_2So_4	—7 to —28	—15	—9	—19
For Na salicylate	2.5 to —10	—8	—7	—7

The evidence shows that the values observed on non-protein colloids lie well within the range of the protein electromotive force in every instance.

A similar coincidence was observed with various other electrolytes, particularly with methylenblue and other dyes. In all these cases, agar agar and the other non-protein colloids exhibited electromotive forces resembling closely those of protein cells.

Gelatin and agar agar exhibit different electromotive forces under certain circumstances, *viz.*, in regard to the effect of the concentration with addition of acid and alkali, as has recently been pointed out by Michaelis.[5] (This point will be taken up by a subsequent publication.) In this respect, however, gelatin is far from being a model to biological electromotive forces, as will be shown later.

Conclusion: As far as gelatin or other proteins may serve as a model for bioelectric currents, their action is identical with that of agar agar, starch paste or kaoline. This is a complete report.

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[1] Hober, R., *Z. Physik. Chemic.*, 1924, cx, 142.

[2] Matsuo, T., *Pflüger's Archiv.*, 1923, cc, 132.

[3] Mond, R., *Pflüger's Archiv.*, 1924, cciii, 247. Also see Fujita, A., *Biochem. Z.*, 1925, clxii, 245.

[4] Deutsch, W., *Pflüger's Archiv.*, 1925, ccix, 675.

[5] Michaelis, L., *Naturwissen Schfaten,* 1925, xiv, 33. See also Fujita, A., *Biochem. Z.*, 1925, clxii, 245.

ADDENDUM.

"Measurements of Fatal Doses of Chloroform in the Brains of White Rats," Vol. XXIV, pp. 340-341. The heading of the second column should read "Chloroform in brain tissue, cm. per gm," instead of cc. per gm.

SCIENTIFIC PROCEEDINGS.

Peking Branch.

Peking Union Medical College, January 13, 1927.

3418

The Effects of Synthetic Diets on Fertility and Lactation.

ERNEST TSO.

From the Department of Medicine, Peking Union Medical College, and the Laboratory of Physiological Chemistry, Yale University.

The existence of a dietary complex essential for reproduction (vitamin E) first described by Evans and Bishop,[1] now corroborated by Sure,[2] Mason[3] and Matill and his associates,[4] has not been generally accepted. Evans and Bishop found that on a synthetic diet composed of casein 18, corn starch 54, lard 15, milk-fat 9, salt mixture 4, plus a daily ration of 0.4 to 0.6 gm. of yeast, rats were for the most part sterile in the first generation, and wholly so in the second. This deficiency is corrected by the addition of lettuce, wheat germ, egg-yolk, meat, and to a less extent milk-fat, or a small quantity of a fat-free concentrate prepared by extraction from wheat germ oil (Evans,[5] and Sure[6]). On the other hand, Nelson, Heller and Fulmer,[7] using a synthetic diet consisting of casein 18, salt mixture 3.7, filtered butter fat 5, yeast 1-8 and dextrine up to 100, encountered no difficulty in securing litters, although only a small percentage of the young could be reared. Likewise, Hogan, Harshaw[8] employing synthetic rations containing casein 20, milk-fat 10, cod liver oil 5, yeast 6, salts 4, corn-starch 50-55 and agar 2-5, reported no sterility in their rats, but rather failure in lactation.

From data accumulated for the past two years in this and in Professor Mendel's laboratory in New Haven, (which are summarized in Tables I and II) and from a review of the more recent

TABLE I.

Synthetic Ration Used.

Ration	Casein	Cornstarch	Salts*	Yeast	Butter-fat	Lard	Cod liver oil	Mixed extract	Yeast extract	Soy bean ash	Fresh lettuce
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	20	41	5	10	9	15	—	—	—	—	—
2	20†	59†	5	10†	5	1	—	—	—	—	—
3	20†	58†	5	10†	5	—	—	2	—	—	—
4	20†	59†	5	10†	5	—	—	—	1	—	—
5	20	59	5	10	5	—	1	—	—	—	—
6	20	59	4.5	10	5	—	1	—	—	0.5	—
7	20	59	5	10	5	—	1	—	—	—	10‡
8	Stock breeding diet: steamed bread, milk, green vegetables, wheat bran and, occasionally, meat.										

*McCollum's salt mixture 185.
†Extracted with absolute alcohol.
‡10 grams to each adult rat per day.

TABLE II.

Fertility and Lactation Record on Diets Exhibited in Table I.

Ration	Number females	Advanced pregnancies terminated by death	Number fetuses found	Number of litters	Total young born*	Young survived beyond 5th day	Young weaned at age of 4 weeks.	Number males on same diet
1	11	0	—	1	5	0	0	6
2	19	1	9	25	187	140	36	12
3	8	1	10	12	91	73	48	4
4	6	0	—	10	77	52	8	4
5	12	6	47	5	32	14	0	5
6	10†	3	24	4	26	15	2	5
7	5	0	—	4	30	27	27	2
8	4	0	—	4	38	36	36	4

*As counted on second day after birth.
†Not including two mother rats that died soon after birth of young.

literature, it seems clear that what appears to be contradictory evidence regarding the existence of vitamin E is only apparently so. On Ration 1, young rats grew more or less normally but were practically sterile; on Ration 2 they reproduced repeatedly. Thus 5 per cent butter-fat in Ration 2 is apparently an adequate source of vitamin E, provided the caloric value of the diet is not raised too high, as in Ration 1. That 9 per cent butter-fat in a high lard diet is not conducive to the production of young is also in harmony with the more recent work of Nelson and his associates[9] who are opposed to the vitamin E theory. A high fat diet, *per se,* is not deficient for reproduction as shown by Evans and Bishop,[1] who secured litters when all of the 24 per cent fat in the diet is supplied in the form of butter-fat. More recently Kennedy and Palmer[10] found that crisco (hydrogenated cotton seed oil), replacing lard in a high fat diet, allows the production of young. Finally, a more definite test for the existence of vitamin E in butter-fat has been shown by Matill and associates,[11] and Sure,[2] who found that rats fed on synthetic diets containing cod liver oil in place of butter-fat were sterile.

The effects of synthetic diets on lactation are more clearly defined. All investigators are agreed concerning the striking failure of rats to rear their young when they are fed certain synthetic diets. This is chiefly due to deficiency in lactation, for the majority of the young lived from a few days to several weeks, and finally died in an emaciated condition before they could be weaned. This is strikingly true in the case of rats fed on Rations 2, 3 and 4.

It was first thought that the casein, starch, and yeast used in synthetic diets might be carriers of a dietary complex necessary for lactation as well as reproduction. The effect of feeding Ration 3 as contrasted with Ration 2, corroborates this view. A much higher percentage of young were reared on a synthetic diet with the addition of the mixed extract than without it. The extract was prepared by treating separately, casein, starch, and yeast, with absolute alcohol at boiling temperature under a reflux. The alcohol was filtered and the filtrate concentrated over a steam bath to a thick brownish liquid. The addition of 2 per cent of the mixed extract, as in the case of Ration 3, introduced several times more of the substance than was originally present in an unextracted ration. The addition of 1 per cent of yeast extract alone, as in the case of Ration 4, proved, however, ineffective. Subsequent experiments with commercial casein, corn starch and Northwestern yeast powder (Ration 5) also failed to demonstrate any appreciable difference between these and extracted products as far as lactation is concerned. Ration 6 was suggested by the work of Daniels and Hutton,[12] who found that the sterility of rats fed on milk diets could be relieved by supplementing the milk diet with soybean ash. The addition of soybean ash to a synthetic ration (Ration 6) exerted no favorable influence on lactation. In marked contrast, lactation was very successful when the rats were fed, in addition to the synthetic diet, 10 grams of fresh lettuce daily, as in the case of Ration 7. It seems evident that lettuce contains something which promotes not only fertility, as shown by Evans and Bishop and others, but also promotes lactation.

All the experiments were started on albino rats 3½ to 4 weeks old, weighing 40 to 50 gm. and were carried out in three series, each at a different time. Thus, Rations 1, 2, 3, and 8 constituted the first series, Rations 2, 4, 5, and 6 the second, and Rations 5, 6, 7, and 8 the last series. In each series except the second, two sets of controls were employed, one with litter mates fed on either Rations 2 or 5, and the other with litter mates fed on Ration 8, which is a stock breeding ration. It seems strange that such a striking number of advanced pregnancies were terminated by death of the animals as in the last series of experiments. There are also other observations which are not brought out in the tables. Although a few rats on Rations 2 and 3 reproduced repeatedly (4 or 5 litters to each rat) several second generation animals raised on the same diet were sterile. Again, the feeding of a number of rats, particularly those on Ration 4, was discontinued before they were given the opportunity to have a second or third litter. It was further observed that the rats fed on the high lard diet did not grow as normally as those on 5 per cent butter-fat rations, and the latter not as vigorously as litter mates raised on a stock breeding diet.

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[1] Evans, H. M., and Bishop, K. S., *Science*, 1922, lvi, 651.

[2] Sure, B., *J. Biol. Chem.*, 1923, lviii, 681.

[3] Mason, K. E., *Proc. Nat. Acad. Sci.*, 1925, xi, 377.

[4] Matill, H. A., Carman, J., and Clayton, M., *J. Biol. Chem.*, 1924, lxi, 729.

[5] Evans, H. M., and Bishop, K. S., *J. Am. Med. Assn.*, 1923, lxxxi, 889.

[6] Sure, B., *J. Biol. Chem.*, 1924, lxii, 371.

[7] Nelson, V. E., Heller, V. G., and Fulmer, E. I., *J. Biol. Chem.*, 1923, lvii, 415.

[8] Hogan, A. G., and Harshaw, H. M., *J. Met. Res.*, 1924, v, 111.

[9] Nelson, V. E., Jones, R. L., Heller, V. G., Parks, T. B., and Fulmer, E. I., *Am. J. Physiol.*, 1926, lxxvi, 325.

[10] Kennedy, C., and Palmer, L. S., *Am. J. Physiol.*, 1926, lxxvi, 316.

[11] Matill, H. A., and Clayton, M., *J. Biol. Chem.*, 1926, lxviii, 665.

[12] Daniels, A. L., and Hutton, M. K., *J. Biol. Chem.*, 1925, lxiii, 143.

3419

Considerations on the Relationship of the Reticulo-Endothelial System to Kala-Azar.

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From the Department of Pathology of the Peking Union Medical College.

These experiments attempted to analyze more closely the nature of the characteristic cellular reaction occurring in the tissues in kala-azar. The hamster *Cricetulus griseus,* a variety of Chinese field mouse, which has been shown to be highly satisfactory for this purpose, was used.[1]

Our first observations were upon the blood obtained by splenic puncture from both normal and infected animals. Differential counts upon preparations supravitally stained with neutral red and janus green by Sabin's technique were made from 20 normals, and 20 infected experimentally with kala-azar, from 10 to 12 weeks.[2] At this time the infection is always well established and the lesions extensive.

The results of these counts are given in the following table.

TABLE I.
Differential Counts Upon Supravitally Stained Splenic Blood.

Type of cells.	Average count of 20 normal Hamsters.*	Average count of 20 Hamsters infected with Kala-azar.*
	Per cent	Per cent
P. M. N.	2.59	2.87
P. M. E.	0.93	0.41
P. M. B.	0.02	0.02
Monocytes	1.70	1.84
Reticulo-endothelium (clasmatocytes)	1.07	6.21
Lymphocytes	91.74	78.93
Unidentified	1.95	9.72

*No. cells counted: 1000 to each animal.

By this method one has little difficulty in identifying practically all of the cells seen in preparations made from normal animals. The great increase, in unidentified cells in infected animals, is caused by the increase in number of definite type of small, mononuclear cell which is very actively motile, and which never has been observed to contain kala-azar bodies. As yet we are uncertain as to the exact identity of this cell, but it clearly seems to have nothing directly to do with the propagation or destruction of the Leishmann-Donovan bodies, and in the tissues, forms no specific part of the lesions. The distinction between monocytes and reticulo-endothelial cells of clasmatocytes was based upon their characteristic receptive staining with neutral red, the monocyte showing a rosette of the dye opposite the "Hof" of the nucleus, while the clasmatocytes of the splenic reticulo-endothelial system take up the stain in large, irregular globules.

Leishmann-Donovan bodies, which stain slightly with neutral red, were found in practically all of the reticulo-endothelial cells of the infected animals, but were never noted in the cells having the characteristic morphology of monocytes. Such supravital preparations are generally swarming with extracellular parasites, which have been liberated by the trauma of the splenic puncture. By a few moments careful observation, one sees the neutrophilic leucocytes quickly phagocytize many of these free kala-azar bodies. Films made from the splenic blood and stained with Wright's stain show that practically all of this phagocytosis takes place upon the slide, for only occasionally are neutrophiles seen which contain parasites.

Many observations made upon the peripheral blood show but little difference between the normal and infected animals. Parasites were found in the latter in almost negligible numbers, and then only in neutrophilic leucocytes.

After these studies were completed each animal was immediately killed and an autopsy performed. Carefully prepared sections show that the parasites are always intracellular, as has been previously noted. For further description of the lesions the reader may refer to a recent paper by Meleney and the accompanying literature.[3]

Upon studying the sections of an animal infected with kala-azar, one is immediately struck with the similarity of the lesions, caused by this disease, to the changes produced in the tissues of experimental animals by repeated injections of India ink. The idea that we may be dealing with the same fundamental cellular reaction in both instances soon suggested itself.

A series of hamsters infected with kala-azar were given small injections of India ink intravenously, and the animals killed on the following day. Sections showed that lesions in the various organs were limited to the reticulo-endothelial system which had undergone tremendous hyperplasia. Practically all of the cells containing kala-azar parasites likewise contained granules of ink, and very few ink-containing cells, which did not also contain parasites, were seen. In another series, which first received India ink and was then infected with kala-azar, the same picture was seen after the infection was well established.

In studying the skin of these animals many large cells filled with Leishmann-Donován bodies were seen in the subcutaneous tissue and in all layers of the skin as well. These cells, of course, cannot phagocytize ink-granules injected intravenously, but when a small amount of ink was injected subcutaneously they immediately engulfed great quantities of it, as did all of the other parasite-containing cells in the various organs. Supravital stains upon small bits of subcutaneous tissue spread out upon the slide showed these cells to be clasmatocytes. These cells are the wandering cells of the reticulo-endothelial system and possess many, if not all, of the properties of the Kupffer cells of the liver and reticulo-endothelium of the spleen, bone-marrow, lymph glands and other organs.

We should like to emphasize the wide extent of the lesions in the skin and subcutaneous tissues of the hamster in kala-azar, which we feel has not been sufficiently recognized. The following illustrations are good examples of sections of skin taken at random from any heavily infected case. Clasmatocytes loaded with parasites in the skin and subcutaneous tissue thus cover the animal with a thick layer of infected cells. It is, therefore, surprising that so many attempts to transmit kala-azar from hamster to hamster by various biting insects have failed.

Summary. Supravital staining of the blood obtained by splenic

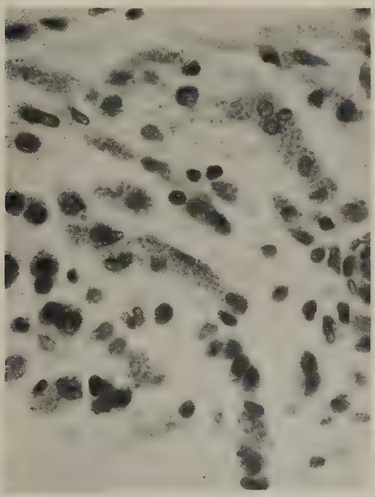

FIG. 1.

Section of skin of hamster infected with Kala-Azar. Several clasmatocytes containing Leishmann-Donovan bodies can be seen in the cutis. Hematoxylin and eosin x 680.

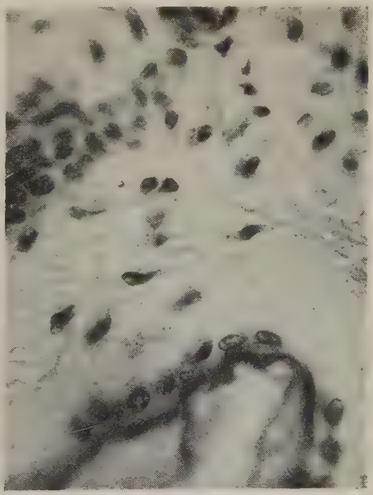

FIG. 2.

Section of subcutaneous tissue of the same animal showing many clasmatocytes loaded with Leishmann-Donovan bodies. Hematoxylin and eosin x 680.

puncture of hamsters infected with kala-azar shows that the large phagocytic cells, which form the characteristic lesions of the disease and contain the kala-azar parasites, have the staining qualities of reticulo-endothelium.

By marking out the cells of the reticulo-endothelial system of animals infected with kala-azar with intravenous and subcutaneous injections of India ink, one sees that the distribution of the lesions and of the parasites is practically limited to this system.

Attention has been called to the nature and wide extent of the lesions in the skin and subcutaneous tissue.

This is a preliminary report.

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[1] Young, C. W., Smyly, H. J., Brown, C., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 357-359.

[2] Sabin, F. R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 277.

[3] Meleney, H. E., *Am. J. Path.*, 1925, i, 147-168.

3420

Antigenic Character of Denatured Egg Albumin.

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The object of the present study is to determine the effect of denaturation on the immunological properties of egg albumin, and to show the relation between the albumins denatured by different methods.

The agents of denaturation used in our study are dilute acids and alkalies, alcohol and heat. Proteins denatured by these different agents have been variously called acid albumins, alkali albuminates, heat coagulated, and, alcohol coagulated proteins. Superficially these substances are similar, and previous work[1, 2, 3] from this laboratory has shown that they are indeed essentially the same in chemical and physical properties. This we have tried to verify by biological methods.

The acid and alkali albumins were prepared by mixing 1 per cent egg albumin solution with equal volumes of H/10 HCl and N/10 NaOH. After 2 or 3 days the denatured egg albumin was precipitated by neutralization and purified by repeated precipitation. The heat-denatured albumins were prepared by heating 100 cc. of 1 per cent egg albumin solution to which 1 cc. of N HCl or N Na_2CO_3

has been added. The acid or alkali was added to prevent flocculation. The alcohol albumin was prepared by adding 40 cc. alcohol to 50 cc. 1 per cent egg albumin to which 10 cc. of 0.3 N Na_2CO_3 have been added. This solution was allowed to stand over night before using.

In testing the antigenic character we used precipitin and anaphylactic reactions. Both reactions show that the denatured albumin, whatever be the agent of denaturation, is immunologically different from the natural albumin. The albumins denatured by different agents are closely related, though not identical.

This is a preliminary report.

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[1] Wu, H., and Yen, D., *J. Biochem.* (Japan), 1924, iv, 345.

[2] Wu, H., and Wu, D. Y., *J. Biol. Chem.*, 1925, lxiv, 369.

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3421

The Period of Induction in Blood Clotting.

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What are the changes taking place in blood plasma from the time it is drawn until the first fibrin appears? Although this period of apparent inactivity occupies the major portion of the so called "clotting time," it has received very little attention from students of the problem of blood clotting. Our recent work establishes certain facts concerning this latent period, or period of induction.

First, fibrin production is not a continuous process, beginning when blood is drawn, but occurs with sudden onset and rapid completion in only a final small part of the "clotting time." If one oxalates plasma only a few seconds before the first signs of fibrin appear, the clotting is completely inhibited. Under such conditions we find the blood fibrinogen unchanged, so that the final appearance of fibrin as a clot represents its actual time of production. Second, since thrombin acts well in the presence of oxalate, we must conclude that, up to the time of oxalation a few seconds before clotting occurs, there has also been no thrombin formed.

Third, preformed thrombin takes over twice as long to clot citrated plasma as it does to clot this same plasma immediately after recalcification, although calcium has no influence on the action of such preformed thrombin. From this we must conclude that the more rapid clotting in the recalcified plasma is due to the formation of new thrombin to aid the old in the fibrin production. We have shown that such formation of new thrombin in plasma may take place in 10 to 20 seconds, when some agent, such as preformed thrombin or tissue fibrinogen, is acting to start a removal of the blood fibrinogen from the plasma. Evidently, once prothrombin is free in the plasma, its activation to thrombin is only a matter of seconds. Therefore in the normal clotting of plasma there can be no free prothrombin up to within a short time preceding the appearance of fibrin.

The latent period preceding fibrin formation seems, then, to be concerned with changes that lead to a liberation of the prothrombin in the plasma. Our work has shown that a variety of agents tending to hasten dissociation, such as the electric current, X-rays, rise in temperature, dilution with water or saline, and the presence of tissue fibrinogen, will all act to shorten this latent period, although not affecting the rate of fibrin production in most instances. The conclusion seems justified that such dissociation is the essential factor in the liberation of prothrombin, and that the speed of this change determines the length of the latent period and thus of the clotting time. Once such dissociation is accomplished, the real clotting reactions take place with great rapidity.

This is a preliminary report.

3422

The Action of Pseudoephedrine Upon the Kidney.

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It has been shown[1] that pseudoephedrine has a blood pressor effect about half as great as ephedrine, and that its effect on the peripheral vessels is opposite to that of ephedrine. Pseudoephedrine dilates the peripheral vessels.[2] Hence it was thought possible that pseudoephedrine might act as a diuretic.

A series of experiments upon 25 dogs was undertaken to see if the dilation of the peripheral vessels applied to the gross effect of pseudoephedrine upon the kidney in the intact animal; moreover whether there was an increase in the secretion of urine, and its relationship to blood pressor effects. The effects of repeated injections were also studied.

Luminalized dogs were treated intravenously with doses of pseudoephedrine hydrochloride ranging from 1 to 11.8 milligrams per kilo. The carotid blood pressure, kidney volume, and urine flow were all carefully observed by ordinary methods involving no special technique. It was found that injections of one or two milligrams of the drug cause a rise in blood pressure of 8 to 22 mm. Hg., lasting 6 to 40 minutes. Larger doses of 5 to 11 milligrams cause a fall in blood pressure of 16 to 22 mm. Hg. Intermediate doses and repeated injections of small doses eventually cause a fall in blood pressure, which may be preceded by injections causing a primary fall of a few mm. and then a rise of blood pressure. A second injection of an average dose of 4 mgm. produced as great a fall as 35 mm. Hg.

The kidney volume follows the blood pressure tracing very closely. A rise in blood pressure causes an increase in kidney volume and a fall is followed by a contraction of the kidney. In one case the denervated kidney showed a decrease in kidney volume while the blood was decidedly increased.

The urine flow was increased in one case only. All other experiments, after a rise in blood pressure and an increase in kidney volume, showed no change in the rate of urine secretion, and whenever there was a fall in blood pressure the urine decreased.

Perfusion of the isolated kidney with Tyrode's solution showed that

1. The vein flow is lessened by concentrations up to .05 per cent. In concentrations from 0.1 to 0.5 per cent of pseudoephedrine the vein flow is increased.
2. Continued perfusion of 0.1 per cent, in 62.5 per cent of the trials causes a contraction of the kidney vessels with lessened output.
3. With 0.01 per cent the urine flow is decreased following a curve consistent with the lessened flow of fluid in the contracted kidney vessels.
4. With 0.5 per cent the urine flow is decreased, there is albumen present. There is indicated severe damage to the kidney which terminates in anuria.
5. In two out of four trials when there was contraction of the kidney vessels, the urine flow increased. This was also observed as a transitory phenomena in a great many trials, indicating that in certain concentrations pseudoephedrine stimulates the secretion of urine, which is usually counteracted by the lessened venous flow.

This is a preliminary report.

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[1] Read, B. E., and Loo, C. T., *China Med. J.*, 1926, xl, 1144.

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Iowa Branch.

State University of Iowa, February 2, 1927.

3423

On the Presence of Aluminum in Blood.

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The question of the absorption of aluminum by the blood and its presence in other body tissues is one of considerable interest. A few figures have been reported for the dog, but they possess little value for the reason that the methods used were inadequate to estimate the very small amounts of aluminum found.

Several very delicate color reactions for aluminum have recently been described. After considerable effort we have been able to utilize one of these reactions, that with aurin described by Hammett and Sottery,[1] for the estimation of small amounts of aluminum in biological material containing iron. Since the amounts of iron left by the ordinary means of separation give a color very similar to that of aluminum, it must be removed more completely. The presence of iron has, however, been utilized to advantage in carrying down aluminum in the first precipitation. For this reason it is necessary to add iron when it is absent.

The method employed for blood is carried out briefly as follows: 5 cc. of blood are digested in a large Pyrex test tube over a micro burner with 2 cc. of sulfuric acid and 3 cc. of perchloric acid, until oxidation is complete. This requires less than 15 minutes.

The digestion mixture is diluted with a little distilled water and transferred quantitatively with washing to a smaller Pyrex test tube, the volume being made up to about 15 cc. One cc. of saturated ammonium acetate is now added and the solution made alkaline with conc. ammonium hydroxide. The tube is placed in a vigorously boiling water bath until the excess of ammonia is expelled. The precipitate, which at first has a tendency to rise, will settle out on shaking. Centrifuge and decant, testing the supernatant fluid with aurin for complete precipitation of aluminum. Dissolve the precipitate in 1 cc. of 6 N hydrochloric acid and dilute with 10 cc. of water, add 1 cc. or glacial acetic acid and 5 cc. of 6 N sodium hydroxide (prepared from metallic sodium), mix and let stand 30 minutes. Throw down the iron precipitate in a centrifuge and decant into a Nessler tube graduated at 50 cc., add 2 cc. of 6 N hydrochloric acid and 1 cc. of glacial acetic acid. Dilute to about 38 cc. with water and add 5 cc. of a 1 per cent solution of the ammonium salt of aurin tricarboxylic acid. Let stand 10 minutes and add 5 cc. or a slight excess of 10 per cent ammonium carbonate in ammonium hydroxide (1 to 2). Make up to 50 cc. Compare against standards of 0.01, 0.02, 0.03, etc., mg. of aluminum containing 3 cc. of 6 N hydrochloric acid, 5 cc. of 6 N sodium hydroxide and 2 cc. of glacial acetic acid. Dilute the standards and develop the color the same as for the unknown.

Analyses have been made on the blood of 13 miscellaneous hospital cases. Repeated analyses have also been carried out on pig's blood. In all instances the amount of aluminum was less than 0.2 mg. per 100 cc. When larger quantities of blood (10 to 30 cc.) were used the results appeared to be somewhat lower. Excellent recoveries of added aluminum have been obtained.

This is a preliminary report.

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[1] Hammett, L. P., and Sottery, C. T., *J. Am. Chem. Soc.*, 1925, xlvii, 142.

3424

Respiratory Function of the Swimbladder in Lepidosteus.*

GEORGE E. POTTER. (Introduced by O. M. Helff.)
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Many investigators have contributed evidence pointing to the probable origin of air-breathing vertebrates from certain physostomous fishes, which apparently have some organ capable of supplementing respiration in times of need. Certain Ganoids and Dipnoians have been credited with this transition of vertebrates from water to land.

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* This work was done under the supervision of Dr. Frank A. Stromsten.

From anatomical data alone, some authors have declared the swimbladder to be a respiratory organ in several genera of fish, including Lepidosteus. It has been observed that Lepidosteus and a few others apparently do inhale atmospheric air,[1, 2] but so far as the writer knows, there exists no proof that this inhaled air is carried into the swimbladder, which would be absolutely necessary if this organ is to be considered an organ of respiration. The existing hypothesis that the swimbladder has such a function is based upon the following facts: (a) that it has a cellular, spongy appearance internally,[3] (b) that blood is distributed to a rich capillary network in its walls, then collected and carried directly to the heart,[4] (c) it has a connection with the pharynx by the *ductus pneumaticus* and glottis and (d) that apparently the animal gulps air into the mouth.[1]

In this article it is shown experimentally that Lepidosteus actually inhales atmospheric air into the swimbladder, where it serves in supplementing the respiration, both normally and to a much greater extent when the oxygen content of the water is low.

The method of procedure has been: (1) to carry on experiments with normal animals in deoxygenated water, allowing part of them to come to the surface for air, and confining others below the surface; (2) to inject melted paraffin through the glottis into the swimbladder of live animals, and run them against controls in normal and deoxygenated water; (3) analysis of gas from the swimbladders of the fish under various conditions, immediately following inhalation of air and again in the interval between inhalations, and (4) analysis of air from above deoxygented water (covered with a film of paraffin oil) in an air tight chamber where an animal is living.

Normal animals live 20 days in a small amount of deoxygenated water if allowed to come to the surface and gulp in air, but they die in 5 or 6 hours if confined below the surface. Control animals live for days with no apparent discomfort when confined by a screen, if a current of oxygenated water passes through the container. This seems to show without doubt that there is supplementary respiration. The average gram hour rate of consumption of oxygen by the fish has been worked out, and shows that the volume of the swimbladder and the normal rate of inhalations is enough to supply sufficient air for the needs of the body.

The paraffin injected animals die in 4 to 8 hours when placed in deoxygenated water while normal control animals live for days in the same water and other injected controls are able to live in normal tank water for at least 10 days. The opercular movement of these animals is about one-third faster than the normal. The injected fish were autopsied and showed a good plug of paraffin in the swimbladder, so that air could not be drawn in. This would indicate that the swimbladder is the organ used for supplementary respiration.

Gas was drawn from the swimbladder with a sampling tube, the canula of which was inserted through the glottis, and analysed for oxygen and carbon dioxide with a Haldane apparatus. Analyses from normal animals in normal tank water show: immediately following inhalation (averages) 7.2 per cent oxygen and 1.59 per cent carbon dioxide; in interval between inhalation, 3.78 per cent oxygen and 2.44 per cent carbon dioxide. Analyses immediately following inhalation in animals which had been disturbed, and were very active, showed 8.03 per cent oxygen and 2.39 carbon dioxide. Activity of the animal seems to cause the carbon dioxide to pile up. Active animals exposed to air for 30 minutes gave the following gas analysis; immediately following inhalation, 10.06 per cent oxygen and 4.1 per cent carbon dioxide, and in the interval, 4.8 per cent oxygen and 4.7 per cent carbon dioxide. Analyses from animals kept in foul water showed: immediately following inhalation, 8.6 per cent oxygen and 3 per cent carbon dioxide, in the interval, 5.02 per cent oxygen and 5.1 per cent carbon dioxide.

The analyses of the air from above the water where an animal is confined in an airtight vessel also gave results which indicate that the fish uses the air in respiration. Some blood analyses are to be tried later in connection with this work.

This is a preliminary report.

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[1] Wilder, B. G., *Proc. Amer. Assoc. Advance. Sci.*, 1875, xxiv, 151.

[2] Day, F., *Proc. Zool. Soc. London*, 1868, 274.

[3] Hoeven, James van der, *Arch. f. Anat.* (Müller), 1841, viii, 221.

[4] Hyrtl, C. J., *Sitzler. Akad. Wiss. Wien.*, 1852, viii, 235.

3425

Hydrogen ion Concentration and Carbon Dioxide Content of Blood of Parathyroidectomized Dogs.

W. F. WENNER AND EDWARD MUNTWYLER. (Introduced by W. W. Swingle.)

From the Zoological Laboratory and the Biochemical Laboratory, State University of Iowa.

In recent years research in experimental and clinical tetany has led to the view that tetany occurs when the reaction of the blood shifts to the alkaline side. The fact that acid producing salts such as $CaCl_2$ and NH_4Cl causes the tetany symptoms to disappear, and the serum calcium to rise supports this view. Wilson, Stearns and Thurlow[1] report that the pH of the blood on the first day of tetany ranges from 7.6 to 7.75, and that the ingestion of 0.1 to 0.5 gm. of HCl brings relief. Grant[2] states that the symptomatic cure of infantile tetany by the use of NH_4Cl is strong evidence in favor of the view that there is an alkalosis in this condition. Freudenberg and György[3] maintain that tetany is the result of a decrease in ionized calcium, coincident with an increase in phosphate, brought about by a change in the reaction of the blood toward the alkaline side.

Drucker and Faber,[4] on the contrary, found that the pH values and the alkali reserve are no greater when tetany is manifest than when it is cured.

Hastings and Murray[5] determined the CO_2 combining power and CO_2 content in four parathyroidectomized dogs. They report only a slight drop. Measurements of the H ion concentration of the blood by means of a potentiometer were made on a different set of parathyroidectomized dogs. Their measurements were made at room temperature that varied considerably. After applying a temperature correction, calculated by Michaelis, they obtained a pH 7.45 for normal.

With this conflicting evidence it was decided to determine simultaneously the pH and CO_2 content of the plasma and calcium content of the serum. Hydrogen ion concentrations were determined electrometrically, using a Clark electrode and a constant temperature chamber whereby a constant temperature of 38° C. was maintained. Van Slyke's water jacketed, fine bore apparatus,[6] graduated to .01 cc., was used for determining the CO_2 content. Clark and Collip's[7] modification of the Kramer and Tisdall method for calcium determination was employed.

The following table gives the readings of 3 dogs that had been receiving treatment, but at the time were in violent tetany. The remaining 3 animals received no medication. These dogs were bled from the left ventricle before operation and at periods thereafter, when they showed faint symptoms of tetany, and later, when tetany became more marked.

TABLE I.

Animal	Date	Time	pH	CO$_2$ content vol. per cent	Serum Ca. mgm. per 100 cc.	Remarks
Dog A	Nov. 22		7.38	49.4		Violent tetany
Dog B	Nov. 22		7.41	40.		Violent tetany
Dog C	Nov. 22		7.37	38.1		Violent tetany
Dog 1	Nov. 30	1:00 P. M.	7.40	39.		
Adult		3:00 P. M.				Thyroparathyroidectomy
female	Dec. 1	9:45 A. M.	7.40	43.8		Normal
	Dec. 2	10:10 A. M.	7.36	34.3		Slight muscle tremors
	Dec. 3	9:25 A. M.	7.35	34.3		Faint muscle twitching
	Dec. 4					Slight tremors
	Dec. 5	2:00 P. M.	7.46	43.8		Marked tetany Very spastic Respiration normal
	Dec. 6					Found dead in cage
9 kilos	Nov. 30	1:00 P. M.	7.40	42.8		
Dog 2		3:30 P. M.				Thyroparathyroidectomy
Adult	Dec. 1	10:00 A. M.	7.40	45.7		Normal
female	Dec. 2	10:20 A. M.	7.40	47.3		Depressed, no muscle tremors
8.5 kilos	Dec. 3	9:20 A. M.	7.29	31.5		Violent tetany hyperpnea, salivation
		9:30 A. M.				Convulsions. Artificial respiration for 2 min.
		9:35 A. M.	7.09*	26.8		Recovered. Respiration normal, tremors disappeared
	Dec. 4-6					No tetany
	Dec. 8					Died in convulsions
Dog 3	Dec. 20	10:45 A. M.	7.38	43.	11.2	
Adult		2:30 P. M.				Thyroparathyroidectomy
male	Dec. 21					Normal
12 kilos	Dec. 22	9:30 P. M.	7.41	40.9	6.4	Slight twitching
	Dec. 23	8:30 A. M.	7.39	38.1	6.1	Hyperpnoea, very spastic
	Dec. 24	1:30 P. M.	7.29	33.4	6.1	Depressed. No muscle twitching, Hind legs paralyzed. Intestinal hemorrhage
	Dec. 25	3:00 P. M.				Died

* This low pH was probably due to the large amount of lactic acid produced during convulsions. The low CO$_2$ was probably caused by a lack of oxygen.

From these results it is obvious that a condition of alkalosis does not exist at any period following parathyroidectomy in dogs. The fall in CO_2 is not accompanied with a corresponding change in the pH, except in the case of dog 2, where the CO_2 content fell from 31.5 volumes per cent before convulsions to 26.8 volumes per cent after convulsions and the pH dropped from 7.29 to 7.09.

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[1] Wilson, D. W., Stearns, T., and Thurlow, M. DeG., *J. Biol. Chem.*, 1915, xxiii, 89.

[2] Grant, S. B., *Arch. Int. Med.*, 1922, xxx, 355.

[3] Freudenberg, E., and György, P., *Klin. Wchnschr.*, 1922, i, 222, 410.

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[5] Hastings, A. B., and Murray, H. A., Jr., *J. Biol. Chem.*, 1921, xlvi, 233.

[6] Van Slyke, D. D., and Stadie, W. C., *J. Biol. Chem.*, 1921, xlix, 1.

[7] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, lxiii, 461.

3426

Metabolic Rate Differences in Amblystoma Larvae.

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Variations in the growth rates of homoplastic and heteroplastic grafts on Amphibia have been observed by many workers. Harrison,[1] for example, noted the retardation and acceleration of heteroplastic grafts in Amblystoma larvae. Differences in the metabolic rates existing between species would seem to afford a partial explanation, at least, for such results. Tests were designed, therefore, to determine the metabolic rates of 81 Amblystoma larvae involving 4 species of Amblystoma, and the strain commonly spoken of as "Axolotl."

The metabolic rates were based on the oxygen consumption for 4 hours, as determined by the Winkler method. All tests were run at 20° C., while all other factors affecting the metabolic rate, such as food and activity, were controlled. An abbreviated resumé of the results follows:

10 *A. punctatum* averaged a 46.9 per cent higher rate than 10 *A. tigrinum.*

12 *A. punctatum* averaged a 47 per cent higher rate than 12 *A. tigrinum.*

10 *A. punctatum* averaged a 24 per cent higher rate than 10 *A. jeffersonianum.*

10 *A. microstomum* averaged a 104.2 per cent higher rate than 10 *A. punctatum.*

17 *A. tigrinum* (Axolotl) averaged a 3 per cent higher rate than 12 normal *A. tigrinum.*

It is apparent, therefore, that considerable differences in metabolic rate do exist between the four species of Amblystoma larvae tested, the order in point of highest rate being: *A. microstomum, A. punctatum, A. jeffersonianum, A. tigrinum.* Such marked differences, substantiated by biometrical analysis, lead one to the general conclusion that the abnormal acceleration of heteroplastic grafts is due partially, at least, to the influence of a higher metabolic rate than the graft was accustomed to. Conversely, a lower metabolic rate on the part of the host, as compared with that of the donor, would tend to retard the growth of the graft.

The apparent difference of 3 per cent between *A. tigrinum* (Axolotl) and normal *A. tigrinum* larvae is of no significance when the probable error is considered, and the two strains of *A. tigrinum* can therefore be said to possess approximately the same metabolic rate. This fact is of interest, in that the neotonous condition of the Axolotl cannot be explained on the basis of a supposed lower metabolic rate.

This is a preliminary report.

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[1] Harrison, R. G., *Proc. Nat. Acad. Sci.*, 1924, x, 69.

3427

Effect of Some Capillary Active Substances on the Permeability of Collodion Membranes.

EARL R. NORRIS. (Introduced by V. C. Myers.)

From the Biochemical Laboratory, State University of Iowa.

Brinkman and Szent-Györgyi[1] report that various capillary active substances, alkaloids[2] and purine bases (sodium oleate, sodium linoleate, sodium glycocholate, digitonin, Witte's peptone, atropine, pilocarpine, caffeine, strychnine, quinine and morphine) cause permeability of collodion membranes to hemoglobin. Rosenthal[3] states that sodium taurocholate possesses the property of increasing the degree of permeability of semi-permeable collodion membranes to dyestuffs. Clausen has shown the presence of some capillary active substance in blood serum[4] and urine of patients suffering from parenchymatous nephritis (nephrosis) which greatly lowers the surface tension, and reports that the addition of blood serum, urine, or, a water solution of the alcohol soluble portion of the colloidal residue from evaporated urine of these patients, will cause collodion membrane to become permeable to proteins.[5] Grollman[6] finds that the collodion sacs used in his investigation were not rendered permeable to hemoglobin by use of bile salts, sodium oleate, lanthanum chloride, acid or alkali.

The permeability of collodion membranes may be varied by many factors in their preparation and subsequent treatment. It has been shown to depend upon the thickness and the ratio of wet to dry weight of the finished membrane, or, according to Brown,[7] upon the alcoholic index of the membrane.

Membranes were prepared in 2 x 17 cm. test-tubes, using 5 cc. of a solution of 2 gr. nitro cellulose in 100 cc. of alcohol-ether mixture. After drying, the membranes were graded according to the method of Brown. Solutions of various substances were placed in the membranes and the membranes then placed in distilled water, the rate of diffusion of the substance across the membrane being taken as an index of the permeability of the membrane, when calculated as the ratio of the concentration outside divided by the concentration inside the membrane at various times. Using the rate of diffusion of $CaCl_2$ across the membrane as an index of permeability the following results were obtained before and after adding a few capillary active substances.

TABLE I.

Effect of Various Capillary Active Substances on the Permeability of Collodion Membranes.

No.		Time of dialysis	Ratio $\frac{\text{Conc. Outside}}{\text{Conc. Inside}}$ for $CaCl_2$ After dialysis	
			Before addition	After addition
1.	Saponine, 0.001 per cent	2 hrs.	0.775	0.755
2.	Saponine, 0.01 per cent	2 hrs.	0.818	0.820
3.	Saponine, 0.1 per cent	2 hrs.	0.750	0.382
4.	Control (nothing added)	2 hrs.	0.755	0.750
5.	Peptone (Witte's)	6 hrs.	0.166	0.120
6.	Water solution of alcoholic extract of residue from evaporated nephrosis urine	6 hrs.	0.106	0.100
7.	Ditto, twice the concentration of 6	6 hrs.	0.105	0.103
8.	Pleural fluid from nephrosis, P. M.	6 hrs.	0.109	0.102
9.	Control (nothing added)	6 hrs.	0.411	0.410
10.	Control (nothing added)	6 hrs.	0.129	0.110

The above results do not show an increase in permeability due to the addition of capillary active substances. The membranes were unaffected by the substance added except for a marked decrease in measured permeability in the case of 0.1 per cent saponine.

Membranes of the thickness used in this work and having an alcoholic index (Brown) of 98 were found to be very permeable to hemoglobin, those of 94 somewhat permeable and 90 impermeable to this substance. We did not succeed in increasing the permeability of a 94 membrane, or in rendering a 90 membrane permeable to hemoglobin by the use of sodium oleate, bile salts, saponine, peptone, or the blood plasma, pleural fluid, or water solution of the alcoholic extract of the residue from evaporated urine of nephrosis cases.

This is a preliminary report.

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[1] Brinkman, R., and Szent-Györgyi, A. V., *Biochem. Z.*, 1923, cxxxix, 261.

[2] Brinkman, R., and Szent-Györgyi, A. V., *Ibid.*, 270.

[3] Rosenthal, S. M., *J. Pharmacol.*, 1925, xxv, 449.

[4] Clausen, S. W., *Am. J. Dis. Child.*, 1925, xxix, 594.

[5] Clausen, S. W., *J. Biol. Chem.*, 1924, lix, Proc. p. xlv.

[6] Grollman, A., *J. Gen. Physiol.*, 1926, lx, 813.

[7] Brown, W., *Biochem. J.*, 1915, ix, 591; 1917, xi, 40.

3428

Cough as a Factor in Chronicity of Experimental Lung Abscess.

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From the Department of Surgery, State University of Iowa.

In a study of the nature of lung abscess produced experimentally in dogs by various means[1] two characteristics of the disease were found in contrast to that in man. In the first place, the animals did not cough appreciably, even at the height of the infection, and, secondly, the abscess cavities, although in some instances of very large size, healed spontaneously and rapidly. It was felt that the absence of cough had direct bearing upon the obliteration of the cavities, and experiments were planned to test this relationship. Preliminary report of these is given, comparing the course of lung abscess in dogs as it occurred without cough, with that of cases in which cough was stimulated. Here the pulmonary lesions were induced by infected emboli, according to the method of Cutler.[2, 3]

In each of 16 dogs a 1.0 cm. segment of femoral vein was resected and used as container for a lead pellet and a fragment of agar bearing a growth of virulent *Staphylococcus aureus,* both ends of the segment being ligated. This capsule was then introduced into the venous stream and allowed to be carried as an embolus to the lung, where it lodged in one of the lower lobes. X-ray examination of the chest was made immediately after embolus, again at about the 16th day, and in most cases before necropsy, usually 30 to 37 days.

Ten of the dogs served as controls. Of these, 7 received no other interference, and 3 were used as operated controls, tracheotomy being performed in addition to the embolus. Six of the dogs were made to cough from the 2nd week on by one means or another, as follows: In 2, tracheotomy was done and through this opening powdered soap bark (Quillaja), an intense irritant to the respiratory mucous membranes, was insufflated into the trachea three times a day. Each insufflation produced an attack of coughing for 5 to 10 minutes, and at other times the dogs coughed occasionally. One dog was induced to cough more continuously by means of a lead pellet suspended in the trachea. The remaining 3 contracted distemper after being given the embolus, and coughed considerably as result of the bronchitis of that disease.

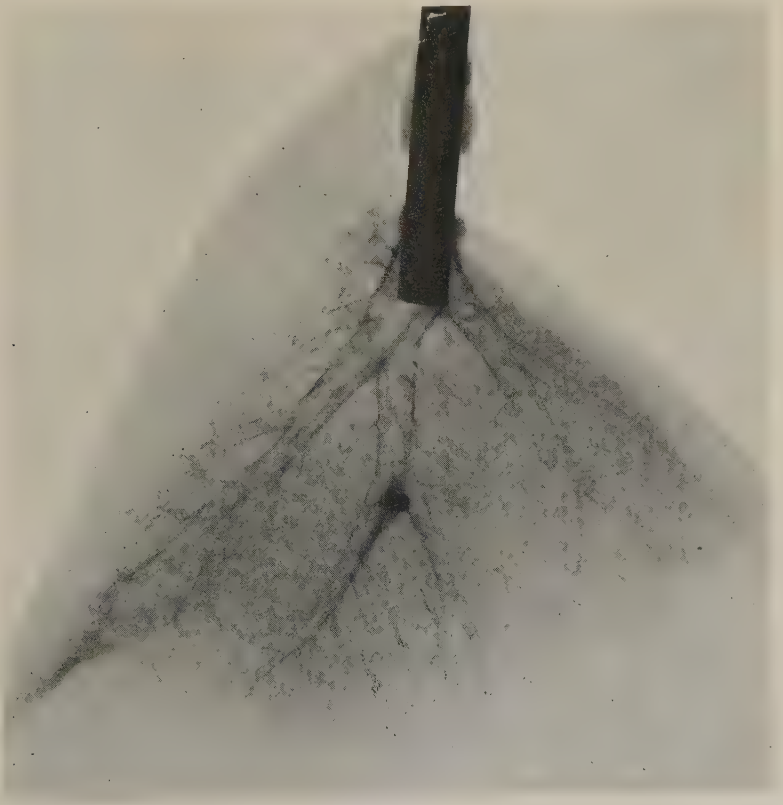
X-ray plates on the 16th day demonstrated cavitation in 5 of the controls, the other 5 presenting only some induration in the region of the pellet marker. Four of the coughing dogs, *i. e.,* 1 with soap-bark insufflations, 1 with the tracheal pellet, and 2 with distemper, showed cavities in the lung field, all greater than those of the controls, The first two being of very considerable size. The other 2 dogs which coughed showed local induration only.

At necropsy, 30 to 35 days, 7 of the controls were found with abscess cavities of size no greater than the pellet which they held. In the other 3 the embolus was still contained entirely within the walls of the pulmonary artery, and the adjacent tissues were intact. Here it was apparent that the infection had been overcome without necrosis of the lung parenchyma. Of the dogs which coughed, the 4 mentioned above had well-defined abscess cavities at necropsy, after 20, 31, 36 and 37 days. They ranged in greatest diameter from 0.9 to 2.2 cm.; 2 were multilocular. Their sizes occurred roughly in proportion to the amounts of coughing taken place. The remaining 2 coughing dogs were found to have the embolus retained within the artery and no cavitation. One of these had died from distemper.

Thus, 70 per cent of the controls and 66 per cent of those which coughed developed pulmonary cavitation as result of the embolus. None of the controls showed persistence of the cavities except as containers of the pellets; while all dogs of the coughing group with cavitation showed larger cavities at the 16th day and persistence to the end of the experiment.

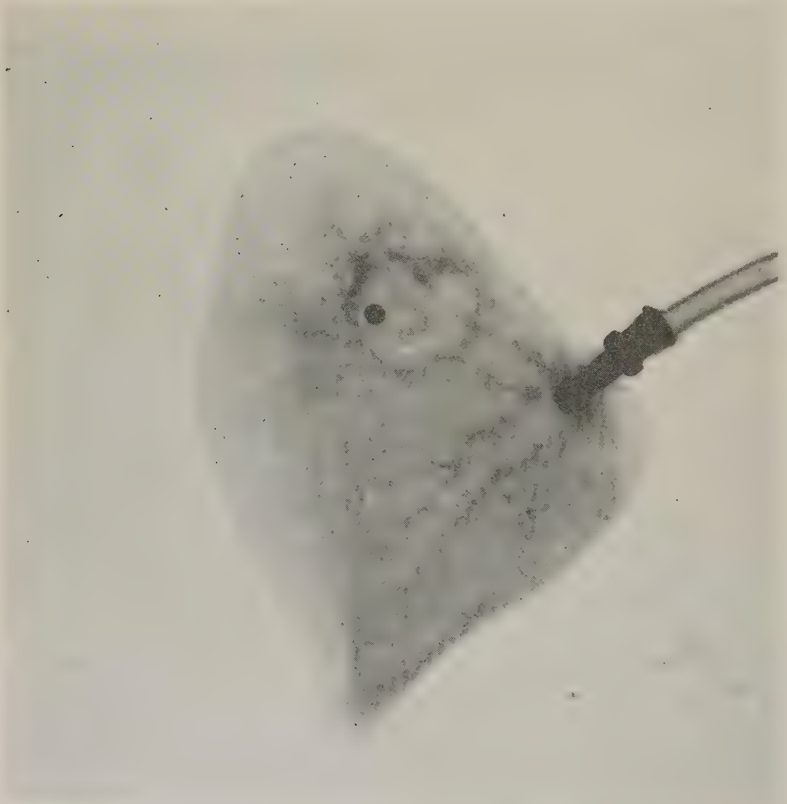
These data indicate, (1) that in dogs an infected embolus in the lung does not always result in abscess, at least where this strain of organism is used, (2) that the disease is characterized by absence of cough and rapid healing, and (3) that when the factor of cough is supplied, the cavities which form may be much larger and persist much longer.

Evidence is not yet at hand as to the means by which the size and chronicity of the abscesses were increased in the presence of coughing. In 2 dogs weakness from distemper doubtless played a part, but that this was a minor rôle is suggested by the fact that another presented a healed lesion, although succumbing from distemper.



Rontgenogram of lung lobe of control dog taken 35 days after embolism, showing healed abscess. Lead pellet indicates position of embolus.

This suggests, also, that merely the jarring and mobilization of the lung by cough was not important. The lesion in this dog, it will be recalled was indurative and not associated with necrosis of the parenchyma; and therein, perhaps, lies the explanation. Where necrosis of the lung parenchyma took place to an appreciable degree, an involvement of at least the finer air passages would be expected, and a communication with the respiratory tract of cavities that formed. In 2 instances gross communication of the cavities with bronchi was demonstrated. The effects of sudden increases of intrathoracic pressure, as in coughing or straining, have been known to lead to rupture of air sacs with the escape of air into the tissues or pleura.[4] Thus, repeated elevations of pressure in coughing transmitted to the abscess cavity from the bronchial tree would probably distend it and impede healing, even make the lesion progressive.



Rontgenogram of lung lobe of coughing dog taken 37 days after embolism, showing abscess cavity containing lead pellet. Cough was stimulated by foreign body suspended in trachea.

For confirmation of these results and study of the mechanism involved, the experiments are being repeated with this embolic type of lung abscess, and also with bronchogenic and interstitially arising types.

This is a preliminary report.

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[1] Van Allen, C. M., unpublished work.

[2] Cutler, E. C., and Schlueter, S. A., *Ann. Surg.*, 1926, lxxxiv, 256.

[3] Holman, E., Weidlein, I. F., and Schlueter, S. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 266.

[4] Sauerbruch, F., *Chirurgie der Brustorgane*, Berlin, 1925, Vol. 2, 714.

Missouri Branch.

Washington University School of Medicine, February 9, 1927.

3429

Skin Reacting Substances in Filtrates of Cultures of Eberthellia Typhi

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If we inject 0.1 cc. of the filtrate of a broth culture of typhoid bacilli intracutaneously in the arm of a human being, there appears in from 12 to 24 hours a reaction similar to that produced by the injection of *Streptococcus scarlatinae* toxin. The reaction fades after 24 to 48 hours, but may leave a slight pigmentation. This reaction has been produced with filtrates of 1, 2 and 5-day cultures, being approximately the same with all. The filtrate was obtained by passing the culture through either Berkefeld W, N or V filters, but usually the N filter was used. Several strains of the organism gave similar results.

The filtrates varied in activity, and we have obtained reactions with dilutions of even one to two thousand. Usually a dilution of 1 to 500 was used.

The reactions produced in normal subjects (who neither had had typhoid fever nor were ever immunized against typhoid fever) are very variable, and while reactions can be produced in all, the area and intensity of the response seem to be individual characteristics. In individuals who had been immunized against typhoid fever, either recently or several years previously, the reactions were similar to and quite as varied as those in the normal subjects. The same statement holds true of individuals who had had typhoid fever some years previous to the injections. No evidence of any ability to inhibit the local reaction was noted in persons who had had typhoid fever, or who had been immunized against typhoid fever.

In individuals having typhoid fever, the reaction to the injection of the filtrate was markedly diminished (as compared with weakly reacting normal individuals) or was negative. During the early period of the disease the reaction might be distinct, in the second or third week the reaction was usually diminished or negative. After convalescence the reaction began again to become positive. The ability of the organism to neutralize the local action of the typhoid filtrate was apparently evanescent, and confined to the actual period of the disease.

At the present time we prefer not to discuss the many possible theories which are suggested by these results, but simply to call attention to the fact that this reaction is apparently not similar or identical with the skin reactions to the so called true toxins, such as diphtheria toxin or scarlet fever toxin.

This is a preliminary report.

3430

Observations on Fat Embolism.

EDWIN P. LEHMAN. (Introduced by E. Graham.)

From the Department of Surgery, Washington University School of Medicine.

Fat embolism, occuring as the result of trauma to fat depots, can be demonstrated clinically and experimentally. Trauma is usually considered the sole cause. However, fat embolism has been found in many non-traumatic conditions. Dogs will survive aseptic injections of cotton-seed oil in amounts per kilo body weight that are twice the amount of fat per kilo body weight in the marrow of the human femur.

It is obvious, therefore, that there must be some other etiology than trauma for some cases of fat embolism. The most logical other source for fat embolism is, of course, the normal plasma emulsion of fat.

Experiments are reported showing that certain artificial emulsions can be broken down by the addition of necrotic muscle extract, peptone and histamine, besides other substances. This breakdown varies in speed and degree inversely with the fineness of the emulsion. It has not been demonstrated in the physiological plasma emulsion of a digestive lipemia.

However, the well-known destruction of this emulsion by fat solvents was applied by the intravenous and mask administration of ether to dogs in the lipemic phase following a fat meal. Ninety-five per cent of animals so treated showed definite fat embolism in the lungs, as compared to 21 per cent of controls which were killed by many different procedures.

It is emphasized that no significance has heretofore been placed on the physical state of the normal fat in the blood-stream. This is the first experimental production of fat embolism by other than traumatic means. The possible relationship of clinical fat embolism to the presence of protein split products in the blood-stream is suggested by the breaking down of artificial emulsions by these substances.

This is a preliminary report.

3431

Action of Aldehydes on Histamine and on Active, Histamine-like Substances Produced by Gas-Bacillus.

ARTHUR ISAAC KENDALL.

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It was shown in a preceding paper[1] that the contraction induced by histamine, or the histamine-like substance produced by the gas bacillus, in a section of guinea pig intestine, or uterus suspended in Tyrode solution, could be relaxed immediately by the addition of 0.1 cc. neutral formalin solution. The most plausible explanation for this phenomenon is a definite chemical reaction between the amino group of the histamine molecule and the aldehyde according to the equation

$$R.CH_2\,CH_2\,NH_2 + H.CHO = R.CH_2\,CH_2\,N : CH_2 + H_2O$$

whereby the ethyl amine group is removed.

It follows that other aldehydes might react in a similar manner, if this explanation be correct.

A series of aldehydes have consequently been tested, using the technique described in the preceding contribution.[1] These comprised, besides formaldehyde, already mentioned, acetaldehyde and its polymer paraldehyde, aldol, glycol aldehyde, glyceric aldehyde and methyl glyoxal, the ketones, acetone and dihydroxyacetone, and acetophenone.

The ketones, and those aldehydes having an oxygen atom attached to the carbon atom next the aldehyde group were without obvious effect. This group included acetone and dihydroxyacetone and the following aldehydes: glycol aldehyde, glyceric aldehyde and methyl glyoxal.

Acetaldehyde, paraldehyde (which probably depolymerizes in Tyrode solution) and aldol, which has the oxygen atom attached to the carbon atom twice removed from the aldehyde group, reduced the contractions promptly. For reasons that are not quite apparent, acetophenone was also very effective in this regard.

These observations are in accord with the hypothesis that certain aldehydes in addition to formaldehyde unite chemically with aromatic amines. They also suggest a pharmacological relationship between the proximity of oxygen upon adjacent carbon atoms and the physiological reactivity of certain aldehydes.

This is a preliminary report.

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[1] Kendall, A. I., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 316.

3432

Pathological Changes in Arteries Following Partial Denervation.

A. H. KERPER AND W. D. COLLIER.

From the St. Louis University School of Medicine.

In a study of the innervation of the arteries of the extremities, segments of the vessels obtained from experimental animals killed at intervals varying from 5 weeks to 3 months following unilateral extirpation of the lumbar sympathetic trunk and the inferior cervical sympathetic ganglion, with or without ligation and section of the femoral and brachial arteries on the opposite side, were prepared by the pyridin silver method. Preparation of the vessels which had been ligated and sectioned still show an abundant nerve supply, though somewhat reduced as compared with that of the normal control vessels. Preparation of the vessels of the sympathectomized limbs show still further reduction in the nerve supply, but still exhibit relatively large bundles of nerve fibers both in the adventitia and the media. The reduction in the number of nerve fibers following sympathectomy is more marked in the arteries of the forelimb than in those of the hind limb. Obviously some of the nerve fibers supplying the arteries of the extremities run along the vessel walls, while others join the vessels via the somatic nerves of the extremities.

Pathological changes in the wall of the arteries following such partial denervation were studied in preparation obtained from a goat about 8 months old, and a kitten. Both these animals had been subjected to extirpation of the left lumbar sympathetic trunk. In the case of the goat, the left inferior cervical sympathetic ganglion was also removed. The kitten was allowed to live 3 months, the goat 6 weeks following operation. Segments of the vessels were fixed in Zenker's fluid, sectioned and stained with Verhoeff's elastic tissue stain.

The more striking pathological changes are found in the partially denervated vessels in the goat, and the most striking changes in the most distal portions of the denervated vascular tree. Less extensive changes, but identical in kind, are found in the partially denervated vessels of the cat. These changes are briefly: atrophy of the muscle fibers with decrease in the thickness of the media and apparent dilatation of the lumen, foci of edematous swelling in the media, particularly just beneath the inner elastic membrane, and considerable fragmentation and disappearance of elastic tissue with a slight proliferation of collagen fibers.

The atrophy of the muscle fibers was not measured directly, but the nuclei show an actual decrease in volume with relative increase in length and decrease in breadth as well as hyperchromatism on the operated side. These changes have been found to be the characteristic changes of depression atrophy (Collier[1]) induced either by depressant stimuli or reduced functional excitation. The latter is apparently the factor in this case following partial denervation, and the muscle change may be interpreted as a relative disuse atrophy. This interpretation is further borne out by the observation of a thinning of the wall and dilatation of the lumen. The dilatation is markedly aided by the fragmentation and partial loss of elastic tissue.

The presence of degenerating edematous areas in the media markedly disturbs the usual uniform, concentric arrangement of the long slender nuclei and the elastic fibers. There is a slight collagen fiber proliferation which suggests an adaptation partially compensating for muscle and elastic tissue degeneration and hints at an eventual definite arterio-sclerotic objective.

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[1] Collier, W. D., *J. Med. Res.*, 1922, xliii, 207.

3433

The Overgrowth of the Connective Tissue Cells in Infected Wounds.

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From the Department of Surgery, Washington University School of Medicine, and the Research Laboratories of the Barnard Free Skin and Cancer Hospital.

In a previous paper[1] the author has shown that clean skin wounds close in large part by a moving forward of the epithelial cells of the epidermis. These cells drag the uninjured skin of the border of the wound across the opening to cover it. This movement takes place early and quickly before there is any extensive growth of the connective tissue cells. Such movement leads to the most perfect restoration of the wounded area. The growing connective tissue cells inhibit this movement of the epidermis.

In infected wounds the picture changes; the connective tissue cells grow in excess of the epidermal cells. The question arose whether these connective tissue cells are more resistant to bacteria than the epidermal cells. A careful study of many infected cultures of mesenchyme, connective tissue cells and epithelial cells has shown that this is not true. The only cells which invade areas of the medium infected with bacteria are the lymphocytes and leucocytes. They appear to be little disturbed by the growing bacteria. All fixed tissue cells are strongly inhibited by all of the growing bacterial cells, so far tested.

Having established this fact, it became evident that other peculiarities of the connective tissue cells or the connective tissue of the skin must allow them to show activity in the presence of bacteria where the epithelial layer cannot become active. In studying the difference between the epithelial and connective tissue cells (fibroblasts) in the tissue culture the author has noted[2] that the connective tissue cells transform the fibrinogen of the exudate of the wound into true fibrin. It was also noted that these cells do not destroy this fibrin except in the areas of the wound where they are crowded, and their environment is sufficiently stagnant for them to grow and divide most actively. While the epithelial cells are also able to transform the fibrinogen into true fibrin, they disolve this fibrin almost as fast as it is formed under the conditions in which they exist in the wound.

In the infected wounds where granulation tissue develops the bacteria grow in the exudate. The connective tissue is the vascular tissue of the wound. In the infected wound fresh fibrinous exudate is being exuded continuously from the blood vessels of the connective tissue layer. The new exudate moves to the free surface of the wound and pushes out the old infected exudate. The epithelial cells dissolve that part of this exudate which comes into contact with them. They contain no blood vessels and supply no exudate. These cells, quite different from the connective tissue cells, must lie in a fluid which is directly continuous with the infected exudate of the wound. The fibroblasts do not liquify the exudate. The new exudate coming from their blood vessels must be forming continuously between them and the infected exudate without.

Such fresh exudate is an active stimulus for the migration and growth of both the connective tissue cells and the epithelial cells. It became of interest to see, therefore, to what extent it might act also as a shield against substances liberated by the growing bacteria. The effect of bacteria of various kinds upon the growth of heart muscle cells *in vitro* have been studied. The cultures were prepared by placing fragments of heart muscle into layers of plasma which clotted about them. Non-liquifying cultures of bacteria growing in such cultures can inhibit the growth of the heart muscle cells for a distance no greater than 0.55 mm. In cultures of tissues containing bacteria which liquify the medium the inhibiting substances diffuse with the ferment which dissolve the clot. They may also diffuse from the liquified zone for a distance of 0.5 mm., but as a rule they do not penetrate the clot so great a distance.[3]

It became evident, therefore, that the sterile fresh fibrin in a wound may be a very effective barrier against the action of bacteria on the cells. It is possible that the overgrowth of the connective tissue cells in many infected wounds is the direct result of the protection of them by this fibrinous layer. The above experiments show that a layer of this substance greater than 0.5 mm. in thickness can protect the cells from the action of any bacteria which does not grow and invade the exudate faster than it is being laid down anew by the blood vessels.

This is a preliminary report.

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[1] Burrows, M. T., *J. Med. Res.*, 1924, lxxvii, 615.

[2] Burrows, M. T., *Anat. Rec.*, 1917, xi, 355.

[3] For other experiments on the diffusion of substances into clotted plasma, see Burrows, M. T., *Am. J. Anat.*, 1926, xxxvii, 289.

3434

Bacteriological Characteristics of Monilia Found in Pernicious Anemia and Certain Other Pathological Conditions.

O. GARCIA, CECILE GARCIA, NANCY BOYCE AND G. O. BROUN.
(Introduced by R. Kinsella.)

From the Department of Medicine, St. Louis University School of Medicine.

The presence of Monilia in the intestinal tract, skin lesions, and bronchial secretions of individuals afflicted with various pathological conditions has long been recognized, particularly through the work of Castellani,[1] Ashford[2] and Fleisher.[3]

Lately, Wood[4] suggested the possible relationship between Monilia and pernicious anemia, and cited some instances wherein he had isolated Monilia, identical with the *Monilia Psiliosis* of Ashford, from the stools of pernicious anemia cases.

We have succeeded in isolating Monilia from 9 out of 10 cases of pernicious anemia. The single case in which we did not find the organism, was in state of remission at the time of the bacteriological examination. Our present paper deals with the bacteriological characteristics of these strains of Monilia found in cases of pernicious anemia as compared to a large number of closely related organisms.

In the past year we have studied over 50 strains of Monilia; of these, 10 were stock strains of known organisms secured from Dr. Fleisher, or from the American Type Culture Collection of Chicago. Nine strains were obtained from active cases of pernicious anemia. The remaining were isolated from cases such as:

Diarrheas	14	Thrush	2
Sprue	2	Diabetes	2
Bronchitis	8	Ca. of stomach	1
Lung Abscess	3	Duodenal ulcer	1
Asthma	2	Cardio-renal	1
Blastomycosis	2	Pyelitis	1
Normal	2		

It was found that there was no difference in the mode of growth on solid (French proof agar) media with the exception of strain No. 6. Here the growth was dry, with a tendency to pile up, resembling very much the growth of tubercle bacilli, in comparison with the smooth creamy white growth of the other strains. All these organisms produce mycelia either laterally or dipping down into the media, with the exception of the above mentioned strain No. 6.

A study of the mode of growth in gelatin shows three types. The first and largest group consisting of those in which the growth was just along the gelatin stab, with no mycelial extensions and no liquefaction of the gelatin; this group would include 4 of the pernicious anemia strains—30, 31, 33, 23.

The second group would consist of those that throw off mycelial extensions into the gelatin, giving the inverted pine-tree appearance. This group includes 5 pernicious anemia strains—Nos. 1, 22, 32, 34, 27.

The third group would consist of those which liquified gelatin. This group includes no pernicious anemia strains.

By far the most interesting results are observed in the sugar fermentation tests. For these we used the following fourteen sugars maltose, dextrose, levulose, lactose, sucrose, inulin, xylose, mannite, raffinose, galactose, arabinose, dulcite, sorbitol and dextrin.

If arranged by the number of sugars in which they produce acid, we find that they fall into eight general groups and sub-groups, A, B, C, etc., according to the sugars they act upon.

According to this classification our ten stock strains fall into the following groups:

No. 39 Monilia Krusei ------------Group II Type B.
No. 37 Monilia Macedonensis ------Group III Type A.
No. 40 Endomyces Albicans -------Group III Type B.
No. 41 Oidium Albicans -----------Group III Type C.
No. 42 Sacchromyces Cerviciae ----Group III Type D.
No. 43 Sacchromyces -------------Group IV Type A.
No. 35 Monilia Albicans ----------Group IV Type A.
No. 36 Monilia Psiliosis 893--------Group IV Type A.
No. 38 Monilia Psiliosis (Ashford)-Group V Type B.
No. 44 Fleishman's Yeast ---------Group VIII Type A.

The organisms from miscellaneous cases fall into the following groups:

No. 2 Blastomycosis -------------Group III Type E.
No. 3 Thrush -------------------Group III Type E.
No. 10 Duodenal ulcer ------------Group III Type E.
No. 18 Blastomycosis -------------Group IV Type A.
No. 25 Pus from jaw -------------Group IV Type A.
No. 45 Ca. of stomach-------------Group IV Type A.
No. 17 Weakness -----------------Group IV Type A.
No. 28 Cardiac ------------------Group VIII Type A.

CHART 1.

Sugar Fermentation Groups

Group	Type	Dextrose	Levulose	Maltose	Galactose	Dextrin	Sucrose	Lactose	Mannite	Inulin	Arabinose	Sorbitol	Xylose	Raffinose	Dulcite	Example Strain No.
I	A	A														6
II	A		A	A	A											8
II	B	A	A				A									39
III	A	A	A		A		A			A						37
III	B	A	A	A		A	A									40
III	C	A	A		A	A	A									41
III	D	A	A	A	A		A									42
III	E	A	A	A	A	A										14
IV	A	A	A	A	A	A	A									36
V	A	A	A	A	A	A	A	A								26
V	B	A	A	A	A	A	A		A							33
VI	A	A	A	A	A	A	A	A	A	A						27
VI	B	A	A	A	A	A	A	A	A				A			21
VII	A	A	A	A	A	A	A	A	A	A	A	A				29
VIII	A	A	A	A	A	A	A	A	A	A	A	A		A		44

Seven strains fall into the pulmonary infection group. Of these 15, 12, 11, taken from cases of bronchitis, and one, 5, from a case of asthma, fall into Group III—Type E. One of bronchitis, No. 24, falls into Group IV—Type A, and two of lung abscesses, 20 and 19, fall into the same groups and types.

Of the ten strains isolated from cases of diarrhea, Nos. 6 and 8 fall into Group I—Type A and Group II—Type A, respectively. Six strains, Nos. 16, 14, 13, 9, 7, 4, fall into the same group, III, and the same type, E. Of the remaining two, No. 21 falls into Group VI, Type A, and the other, No. 29, falls into Group VII—Type A.

The nine strains of pernicious anemia fall into the following groups: Four of them, Nos. 30, 31, 34, 23, fall into Group IV—Type A. Two, Nos. 26 and 23, fall into Group V—Types A and B. The remaining three into Group VI—Type A.

Summary: 1. A study of the sugar fermentation reactions of the strains of Monilia isolated from cases of pernicious anemia, shows that all act upon dextrose, levulose, maltose, galactose, dextrin and sucrose. The action on lactose, mannite and inulin varies with the different strains. These reactions can be used to distinguish these organisms from various stock strains of yeasts. They resemble closely those given by the stock strains of *Monilia psiliosis*.

2. Monilia isolated from other pathological conditions, in general, seem closely related to the pernicious anemia strains in type of growth, and fermentation reactions, although some slight differences are found in many strains.

We take this opportunity to express our appreciation to Dr. Moyer S. Fleisher for his cooperation and encouragement throughout this work.

This is a preliminary report.

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[1] Castellani, A., and Chalmers, A. J., *Man. Trop. Med.*, 1913, pp. 820-32.

[2] Ashford, B. K., *Am. J. Med. Sci.*, 1915, cl, 680.

[3] Fleisher, M. S., and Wachowiak, M., *Am. J. Med. Sci.*, 1924, clxviii, 371.

[4] Wood, E. J., *So. Med. J.*, 1925, xviii, 157-162.

3435

Simultaneous Cholecystography and Determination of Liver Function.

W. H. COLE. (Introduced by E. Graham.)

From the Department of Surgery, Washington University School of Medicine, and Barnes Hospital.

Since the introduction of cholecystography by us three years ago, we have instituted many changes and modifications to enhance its value. A short time ago we substituted phenoltetraiodophthalein for tetraiodophenolphthalein for several reasons. A smaller dose is required, the time of filling and emptying of the gallbladder is shortened, and the blood serum is stained so that readings on the excretion by the liver can be obtained.

The dose of phenoltetraiodophthalein is 0.04 grams per kilo of body weight, and is preferably given in the morning intravenously on an empty stomach. The dose need not exceed 2.5 grams. Lunch should be omitted, but if hunger demands, the diet should be limited to liquids without fats and low in proteins. Water is allowed. Roentgenograms are taken 4, 8 and 24 hours after the injection.

The solution is prepared by dissolving 2.5 grams phenoltetraiodophthalein in 30 or more cc. freshly distilled water, filtered and sterilized in a water bath for 15 or 20 minutes. A specimen of blood is taken before giving the dye and one-half hour afterwards. The blood is allowed to clot, preferably in an icebox and centrifuged. The serum is removed, alkalinized and compared with standards in a manner similar to the Rosenthal modification[1] of the phenoltetrachlorphthalein test for liver function.

The standards are prepared by dissolving 48 milligrams phenoltetraiodophthalein in 100 cc. distilled water. This is used as the 100 per cent solution, and 10 or 12 dilutions are made between 100 per cent and 5 per cent. A small amount of alkali must be added to the standards to keep them from fading. The correct amount for 4 or 5 cc. of standard is one drop of 5 per cent sodium hydroxide for standards between 60 and 100 per cent, 2 drops for standards between 25 and 60 per cent, and 3 drops for standards between 3 and 20 per cent. In arriving at the figure 48 milligrams, we have used the ratio of 1 to 12, for estimating blood volume, as is recognized by Erlanger and others.

Retention of less than 15 per cent of the dye in the blood serum one-half hour after injection of the dye, and less than 4 per cent one hour after injection is considered normal. Retentions of any greater amounts for the specified time are considered abnormal.

The largest amounts of retention have been found in patients having cirrhosis with ascites, and may be as high as 80 to 90 per cent one half hour after injection. Retentions almost as great have been seen in patients having acute hepatitis with cholangitis, usually with slight jaundice. An interesting and almost constant fact has been observed, viz., that a mild retention above normal, ranging between 17 and 35 per cent, is found in cases of cholecystitis. This is probably explained by the practically constant hepatitis accompanying cholecystitis.

Animal experiments are now being conducted to determine the comparative values of phenoltetraiodophthalein and phenoltetrachlorphthalein as agents for determining liver function. In these experiments we have used phenoltetrachlorphthalein in doses of 5 milligrams per kilo as recommended by Rowntree, Hurwitz, and Bloomfield,[2] but phenoltetraiodophthalein in doses of 40 milligrams per kilo because of the advantage, clinically, of obtaining a simultaneous cholecystogram. Satisfactory cholecystograms are not obtained with phenoltetraiodophthalein in doses much smaller than 40 milligrams per kilo. The amount of retention found in the blood of animals with normal livers, as well as those having liver necrosis of varying degree, produced by chloroform anesthesia, would very obviously be less with phenoltetrachlorphthalein than with phenoltetraiodophthalein, when using doses of 5 milligrams per kilo and 40 milligrams per kilo, respectively. Because of the chemical similarity of the two dyes, there is no reason to believe there would be much difference in the time of excretion by the liver, if they were given in equal doses. The average figures for normals after injection of phenoltetraiodophthalein are, as follows: 10 per cent for 15 minutes, 6.6 per cent for 30 minutes, 3.5 per cent for 45 minutes, and 2 per cent for 60 minutes. For phenoltetrachlorphthalein: 9.2 per cent for 5 minutes, 5.5 per cent for 10 minutes, 3.5 per cent for 15 minutes, and 2 per cent for 30 minutes. Chloroform is being used for production of liver injury and readings taken on the second day and continued until the liver has regained its normal excretory power. Only a limited number of experiments have been completed as yet, but, in every case, phenoltetraiodophthalein has shown retention as long or longer after production of the liver damage, as has phenoltetrachlorphthalein and without producing any toxic effects. This factor of greater sensitivity of phenoltetraiodophthalein, produced presumably by the larger dose, would seem to be quite a definite advantage, and has manifested itself in the few experiments conducted so far.

This is a preliminary report.

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[1] Rosenthal, S. M., *J. Pharmacol. and Exp. Therap.*, 1922, lxx, 385-392.

[2] Rowntree, L. G., Hurwitz, S. H., and Bloomfield, A. L., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 327.

3436

Effect of Potassium Iodide and Thyroid Extract on Thyroid Gland of Guinea-Pig.*

S. H. GRAY, F. L. HAVEN and LEO LOEB.

From the Department of Pathology, Washington University School of Medicine.

In a series of investigations on the compensatory hypertrophy of the thyroid gland in guinea pigs, it was found that within the normal gland there was discernible a formation of three layers which differed in (1) the size of acini, (2) the more or less solid state of the colloid, (3) the quantity of this substance and (4) the height or flatness of the epithelium lining the acini. There is usually associated with a greater hardness and a greater quantity of the colloid a greater flatness of the epithelium.[1] This suggested that the character of the epithelium lining the acini may be determined, at least in part, by the condition of the colloid, and in particular by the presence or absence of pressure exerted upon the epithelium by this substance, and by surrounding acini. There was some apparent confirmation of this view in the further observation that, not rarely, the walls separating neighboring acini may be broken through, and that such a change may be followed by the union of the colloid material of both acini. This fact also suggested a pressure effect.

It has been observed by Marine and Lenhart[2] that in certain kinds of goitre, administration of potassium iodide may cause an increase in the amount of colloid of the acini, and reduce the height of the epithelium. More recently several authors have found that in exophthalmic goitre potassium iodide may at least temporarily lead to a reduction in size of the epithelium lining the acini. On the other hand, it has been observed that under certain conditions administration of this substance may intensify the symptoms of abnormal thyroid activity, and in our series, referred to above, it was shown that contrary to current opinion, feeding of potassium iodide to guinea pigs, from which great parts of their thyroid gland had been removed, did not diminish the subsequent compensatory hypertrophy of this organ, but that, on the contrary, in our rather extensive series, it seemed definitely to increase the size of the cells, the number of mitoses, as well as the fluidity and absorption of the colloid. These facts suggested that under certain conditions potassium iodide may exert a stimulating effect on the thyroid.

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* The essential facts and conclusions in this paper were presented at the meeting of the Missouri Branch of the Soc. for Exp. Biol. and Medicine held December 15, 1926.

On the other hand, we found that feeding of thyroid substance prevents compensatory hypertrophy; it makes the colloid more solid, the epithelium lower, and diminishes the number of mitoses, and in certain cases it seems even to cause a decrease in the size of the acini and of the gland. Feeding with anterior pituitary extract has a somewhat similar effect to that produced by thyroid extract, although this effect was less pronounced.[1]

In order to contribute, if possible, to an explanation of this apparently contradictory evidence, we decided to investigate the effect of feeding potassium iodide and thyroid substance on the structure of the normal thyroid gland in guinea pigs. Our main observations are as follows: Feeding potassium iodide to normal guinea pigs does not lead to a marked diminution in the size of the acinus cells within the first 30 days. If there is possibly some diminution in some cases, there are, on the other hand, other cases in which the acinar cells are at least as large as the average cells in the controls or even larger. From about 40 days on, following the feeding with KI, the epithelium becomes lower and gradually the acini enlarge. The colloid now is solid and hard. Mitotic activity of the acinar epithelium is decidedly increased during the first 6 weeks following administration of KI. In a number of cases, mitoses were very numerous in the acinar epithelium of the KI fed animals. On the other hand, the acinar epithelium of the thyroid fed guinea pigs is on the whole relatively low, the colloid is solid, and the number of mitoses is smaller than even in the control series.

It seems to us that it may, perhaps, be possible to reconcile the apparently contradictory evidence as to the effects of potassium iodide and thyroid substance on the thyroid gland by assuming that administration of iodide stimulates the thyroid gland to increased activity, as evidenced by the increased number of mitoses. However, under these circumstances the colloid produced does not leave the acini in a sufficient quantity; it remains largely stored in the gland, and thus it may gradually exert an injurious pressure on the acinar walls, and in particular on the epithelium lining the latter. As the result of these injurious changes, perhaps in association with some other factors, the activity of the gland is gradually inhibited. However, if we set into motion a mechanism which causes the rapid solution of the colloid, and its removal into the vascular system, these secondary injurious influences may be prevented. This may be accomplished through removal of great parts of the thyroid gland

and thus we often find in compensatory hypertrophy of the thyroid gland an increased activity under the influence of potassium iodide. There may be, in addition, still other factors involved in the action of this substance on the thyroid gland. In the case of thyroid feeding, an excess of thyroxin in the circulation prevents the mobilization of colloid in the gland, which remains thus solid, and in addition the thyroxin may perhaps gradually cause an atrophy of the epithelium, as an expression of the inactivity of the gland produced by the excess of the hormone.

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[1] Loeb, Leo, *J. Med. Res.*, 1919, xl, 199; 1920, xli, 481; 1920, xli, 77; *Am. J. Path.*, 1926, ii, 19. Loeb, Leo, and Hesselberg, Cora, *J. Med. Res.*, 1919, xl, 265. Loeb, Leo, and Kaplan, E. E., *J. Med. Res.*, 1924, liv, 557.

[2] Marine, David, and Lenhart, C. H., *Arch. Int. Med.*, 1909, iv, 253.

Southern Branch.

Tulane University, February 10, 1927.

3437

The Effect of Carbon Arc Radiation on Circulation in the Dog.

HENRY LAURENS AND H. S. MAYERSON.

From the Laboratory of Physiology, Tulane University.

Depression of the general arterial blood pressure by radiant energy has been repeatedly demonstrated, although a rise in pressure, during or soon after irradiation, has also been recorded. We have investigated the effects of known amounts of carbon arc radiation on the blood pressure, pulse rate and body temperature of normal adult dogs under standard conditions. A total of 13 experiments on 8 dogs have been completed, 11 with the Majestic Arc, and two with the Pan Ray Arc* as the source of energy. The distribution of the energy irradiated by the two lamps, as determined spectroradiometrically† is approximately, for the Majestic Lamp or "Solarite": ultra-violet 21.9 p. c., visible 43.5 p. c., infra red 34.6 p. c., and for the Pan Ray Arc 15, 59 and 26 per cent, respectively. The total energy of the "Pan Ray Arc" is about 6 times that of the Majestic, being equal to 1 gm. cal. per sq. cm. per minute at 103 cm.

In the experiments with the Majestic Arc the animals were irradiated daily for 1 hour, either on the back or the abdomen, at a distance of 40 cm. from the lamp for periods of from 8 to 33 days. Each dose had a total energy equivalent to 55.44 gm. cal. per sq. cm. In 2 experiments the time of the exposures was increased to 2 hours daily for 8 days. In the first experiment with the Pan Ray Arc, the abdomen of the animal was irradiated for 45 minutes at 1 meter (45.0 gm. cal. per sq. cm.) and the dosage progressively increased

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* The Pan Ray Arc was presented to one of us (H. L.) by its makers, The Atlas Electric Devices Co. of Chicago, to whom it is a pleasure to record here our thanks.

† A portion of the cost of the spectroradiometric apparatus was defrayed by a grant from the Elizabeth Thompson Science Fund.

TABLE I.
Effects following fifth daily exposure.

	Systolic Pressure			Diastolic Pressure			Pulse Pressure			Pulse Rate		
	Before	After	24 hrs. after	Before	After	24 hrs. after	Before	After	24 hrs. after	Before	After	24 hrs. after
Dog A (1)	148	120	148	80	68	86	68	52	62	76	80	100
Dog A (2)	144	124	154	72	93	80	72	31	74	92	100	86
Dog C	168	140	184	121	92	108	47	48	76	88	112	80
Dog D	182	147	174	123	104	115	59	43	59	104	100	116
Dog E	161	142	169	81	75	77	80	67	92	80	84	80
Average	160	134	166	95	86	93	65	48	72	88	95	92

Average p. c. decrease: systolic = 16 p. c.; diastolic = 10 p. c.
Dogs A (1), A (2), C, abdomen exposed. Dogs D, E, back exposed.

during a period of 13 daily exposures. In the second experiment, the animal was exposed to massive abdominal exposures at intervals of 2 or more days. Systolic and diastolic pressure values were obtained by auscultation of the femoral as suggested by Allen.[1]

Abdominal irradiation results in a decrease of 5 to 20 p. c. in the systolic pressure after either the first or second exposure, persisting for about 5 hours, and is followed by a gradual return to normal. The diastolic pressure, in some cases, shows a proportional decrease, in others it rises during irradiation and returns to normal soon after. When the back of the animal is exposed, similar changes are evident after the third or fourth dose. The effects in all cases usually reach a maximum on the fifth exposure (Table I), and re-occur with each subsequent irradiation. With few exceptions, all values tend toward normal levels within 24 hours after each exposure. The pulse rate usually increases during irradiation, but this effect is not consistent. Body temperature shows little variation, slight rises during irradiation disappearing within an hour after the exposure is over. If the daily dosage is progressively increased, the magnitude of the changes is proportional to the strength of the radiation.

Long lasting depression of the blood pressure was obtained in two experiments with white, short-haired animals, where the absorption of radiant energy by hair and pigment was doubtless at a minimum. In one experiment, after the twelfth daily irradiation with the Majestic Arc, the dog showed a decrease of about 20 p. c. and 15 p. c. in systolic and diastolic pressures respectively, the values remaining at this level through 5 subsequent exposures and for 3 days thereafter before returning to normal. The most marked effects were obtained in massive irradiation (½ to ¾ hour at 60 cm.), with the Pan Ray Arc at intervals of 2, 3 and 6 days respectively. After the fourth exposure, the levels of both systolic and diastolic pressures dropped and remained low for over a week and then slowly

TABLE II.
Effects following abdominal irradiation with Pan Ray Arc.

	Systolic Pressure	Diastolic Pressure	Pulse Pressure	Pulse Rate	Rectal Temperature °C.
Normal	171	70	101	85	38.1
Immediately after last irrad.	122	79	43	82	38.0
1 day after last irradiation	124	47	77	118	39.4
5 days after last irradiation	123	61	62	88	38.4
10 days after last irradiation	151	54	97	91	38.7
15 days after last irradiation	152	68	84	84	38.4
20 days after last irradiation	168	67	101	73	38.1

rose to normal on the twentieth day. (Table II.) The pulse rate usually increased during the irradiation (average 20 p. c.), returning to normal soon after. Body temperature remained elevated about 1° C. for two days after the last irradiation.

Control dogs placed behind a screen at the same distance from the lamp while the experimental animal was being irradiated showed no changes in blood pressure, pulse rate or body temperature.

This is a preliminary report.

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[1] Allen, F. M., *J. Met. Res.*, 1923, iv, 431.

3438

Action of Bacilli of Paratyphoid, Dysentery and Metadysentery Groups on Various Starches.

ALDO CASTELLANI.

From the Tropical Department, Tulane University.

The action of certain bacteria on potato starch has been studied, but very little is known on the action of bacteria on the less common starches. For some time I have carried out researches on the action of bacilli of the paratyphoid, dysentery and metadysentery groups on various starches, and on the possibility of using starch reactions in the differentiation of organisms of these groups.

Fifteen starches have been used, *viz.,* arrowroot, barley, bean, canna, corn, ginger, lentil, oat, pea, potato, rice, sago, tapioca, yam, wheat. The so-called soluble forms have been used, but solution is seldom complete.*

Technique. Tubes containing 1 per cent solution of the various starches in peptone water are used, each tube containing a Durham's

TABLE I.

Action on starches of B. paratyphosus A and B. paratyphosus C.

	Arrowroot	Barley	Bean	Canna	Corn	Ginger	Lentil	Oat	Pea	Potato	Rice	Sago	Tapioca	Wheat	Yam
B. paratyphosus B	0	0	0	0	0	A	0	0	0	A	0	A	0	A	A
B. paratyphosus A	0	0	0	0	0	0	0	0	0	0	A	A	A	0	0
B. paratyphosus C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The readings were taken after 3 weeks incubation at 37° C. After 6 weeks, the readings were the same but paratyphosus C produced a slight acidity in tapioca.

* The starches were obtained from the Eli Lilly Co., Indianapolis.

TABLE II.
Action on starches of B. dysenteriae Shiga-Kruse, B. dysenteriae Flexner, B. dysenteriae Y.

	Potatoes	Ginger
B. dysenteriae Shiga-Kruse	0	0
B. dysenteriae Flexner	A	A
B. dysenteriae Y	0	0
B. dysenteriae Duval	0	0

The readings given in the table are those taken after 3 weeks incubation at 37° C.

fermentation tube, or saccharymeters may be used. It must be noted that many starches do not dissolve completely. The tubes are inoculated with the different organisms and placed in the incubator at 37° for 21 days, readings being taken on the 7th day, the 14th day and the 21st day.

I suggested some years ago the term "metadysentery bacilli" to denote a group of bacilli capable of producing dysenteric symptoms in man but culturally different from the true dysentery bacilli (Shiga-Kruse, Flexner, Hiss-Russell). These bacilli are characterized by the following features:

1. Like the true dysentery bacilli they do not produce gas in any sugar.
2. In contrast to the true dysentery bacilli they either produce acidity in lactose and clot milk, or produce acidity in lactose without clotting milk, or clot milk without producing acidity in lactose.

There are several species of the metadysentery bacilli which Chalmers and I grouped into two genera: Lankoides (milk clotted), Dysenteroides (milk not clotted). The two principal species are *B. ceylonensis* A and *B. ceylonensis* B, and my experiments have been carried out with these two germs; the results are collected in Table III.

Discussion and Conclusions. With the strains in my possession it is possible to differentiate by means of their starch reactions be-

TABLE III.
Action on starches of certain bacilli of the metadysentery group.

	Arrowroot	Barley	Bean	Canna	Corn	Ginger	Lentil	Oat	Pea	Potato	Rice	Sago	Tapioca	Wheat	Yam
B. ceylonensis B, Cast. 1907 *B. ceylonensis* B	0	A	0	A	A	A	0	0	0	A	A	0 (decol)	0	A	0
B. ceylonensis A, Cast. 1907 *B. ceylonensis* A	0	0	0	0	0	0	0	0	0	0	0	A	A	A	0

The readings given in the table are those taken after 3 weeks incubation at 37° C.

tween *B. paratyphosus* A, *B. paratyphosus* B, *B. paratyphosus* C; between *B. dysenteriae* Shiga, *B. dysenteriae* Flexner, *B. dysenteriae* Y (Hiss and Russell); between *B. ceylonensis* A and *B. ceylonensis* B.

B. paratyphosus B produces acidity (and often gas) in wheat, ginger, potato, sago; *B. paratyphosus* A does not touch wheat, ginger or potato; it touches sago, rice and tapioca; *B. paratyphosus* C does not touch any starch the first 3 weeks, then may produce acidity in tapioca; *B. dysenteriae* Shiga does not touch potato or ginger; Flexner produces acidity in both; Y does not touch them; Duval does not touch them. It is interesting to note that a strain received from a well known laboratory marked Flexner did not touch potato or ginger; further investigation showed this organism to be inert on maltose and to be highly agglutinated by Y serum. *B. ceylonensis* B produces acidity in ginger, corn, potato, rice; *B. ceylonensis* A produces acidity in sago, tapioca, wheat.

If further investigation should prove that all strains of these various organisms have permanent starch reactions, this might be an additional means for differentiating them.

Addendum. It is well known that different starches differ microscopically, their granules being of different appearance. They differ also in all probability biologically and biochemically, as some starches are fermented by certain microorganisms, other starches by other organisms. The testing of a given starch by means of various organisms assist in the identification of that particular starch.

3439

"Symbiotic Fermentation Phenomenon," Its Use in Differentiation of Microorganisms and Identification of Carbon Compounds.

ALDO CASTELLANI.

From the Department of Tropical Medicine, Tulane University.

The phenomenon called symbiotic fermentation may be defined as follows: "Two microorganisms neither of which alone produces fermentation with gas in certain carbohydrates may do so when living in symbiosis or when artificially mixed." I noted the phenomenon in 1904 and 1905[1]; I observed that ordinary baker's yeast in Ceylon and England, as a rule, consisted of two or more organisms (saccharomyces and bacilli) living in symbiosis, and that baker's yeast *in toto* gas-fermented a larger number of sugars than any of the isolated organisms. In recent years I have used principally pathogenic bacilli. The phenomenon takes place using only certain organisms, one of which apparently must be capable of producing acidity (never gas) in certain carbohydrates.

Examples: I. *B. typhosus* alone does not produce gas in maltose (acidity only); *B. morgani* alone does not produce gas in that sugar (neither acidity nor gas). The mixture *B. typhosus* + *B. morgani* will produce gas.

II. *Staphylococcus aureus* (Ross Institute strain) alone does not produce gas in maltose (acidity only); *B. morgani* alone does not produce gas (neither acidity nor gas); the mixture *Staphylococcus aureus* + *B. morgani* will produce gas.

Liquid media. The phenomenon is easily put in evidence by using the test tubes containing a Durham's fermentation tube or saccharomyters. It is essential that the liquid media (maltose, mannitol, etc.) should be made with sugar-free peptone water, not broth, which as well known usually contains a small amount of glucose.

Solid media. The phenomenon is very evident when solid media are used. The solid sugar media (maltose-agar, mannitol-agar, etc.) are prepared in the usual way, but in the preparation of the agar, sugar-free peptone water must be used, not broth, because broth as a rule contains a little glucose.

When the phenomenon is positive a large number of gas bubbles will be present.

According to the very recent work of Schutze[2] and Harde,[3] the phenomenon is due to one of the organisms causing formation of substances which are gas-fermented by the other. In the case of *B. typhosus* + *B. morgani* producing gas in maltose, this is apparently due to the formation of formic acid from maltose by *B. typhosus;* formic acid is gas fermented by *B. morgani.*

I have found the symbiotic fermentation phenomenon useful in

TABLE I.

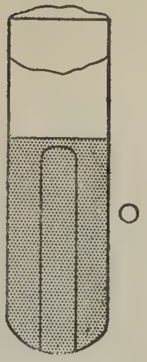
Differentiation between B. dysenteriae Shiga, B. dysenteriae Flexner, B. dysenteriae Y (Hiss and Russell) by means of their symbiotic fermentation with B. morgani.

	Mannitol	Maltose
Shiga + Morgani	0	G
Flexner + Morgani	G	G
Y + Morgani	G	0

Differentiation between Shiga, Flexner and "Y" by means of the symbiotic fermentation phenomenon.

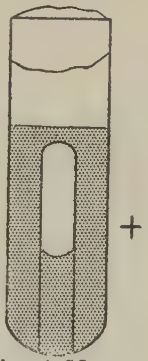
SHIGA.

Mannitol



Shiga + Morgan

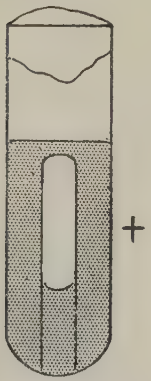
Maltose



Shiga + Morgan

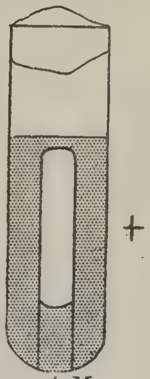
FLEXNER.

Mannitol



Flexner + Morgan

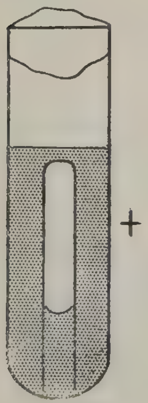
Maltose



Flexner + Morgan

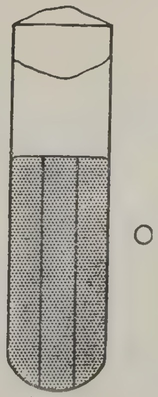
"Y"
(Hiss-Russell)

Mannitol



"Y" + Morgan

Maltose



"Y" + Morgan

the differentiation of certain microorganisms, for instance, organisms of the dysentery group. *Shiga* may be differentiated from *Flexner* and *Y* in this way: *Shiga* + *Morgani* will not produce gas in mannitol; *Flexner* + *Morgani* will produce gas; *Y* + *Morgani* will produce gas. *Flexner* may be differentiated from *Y* in this way: *Flexner* + *Morgani* produces gas in maltose, *Y* + *Morgani* does not produce gas in that sugar.

It may be said in general that the results of the symbiotic fermentation correspond to a great extent to the results obtainable with simple acid fermentation, *viz.*, there will be gas in those carbohydrates in which the organism to be identified will produce—alone—simple acidity; the presence of gas, however, is much more striking than simple acid fermentation, and moreover, it may in certain cases become evident very rapidly while the simple production of acid may be very slow. There has been a great deal of discussion on the point whether *B. dysenteriae Shiga-Kruse* produces acidity in maltose. In the older textbooks it is often described as giving acidity in that sugar; in the modern textbooks, as a rule, as not giving acidity. I have found that the symbiosis *Shiga* + *Morgani* produces gas in maltose, though in small amount, and therefore it is practically certain that *Shiga* must produce acidity in that sugar.

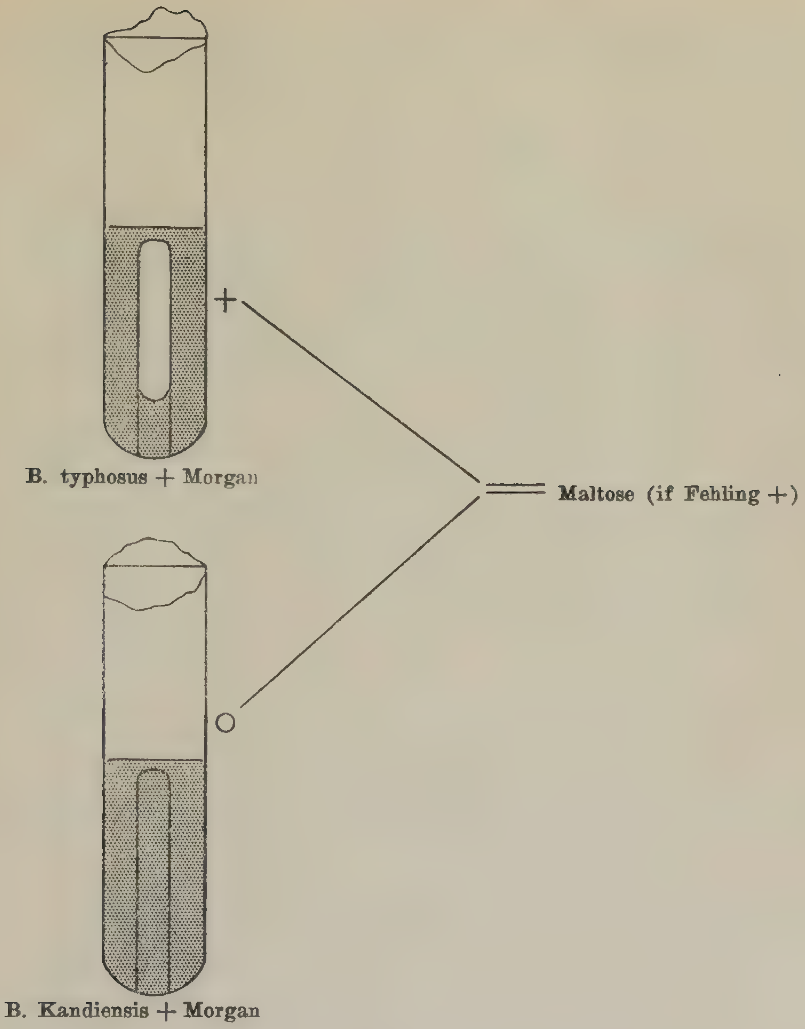
The experiments on this subject will be given *in extenso* in another paper. In this paper I may say that the phenomenon may be made use of in the identification and differentiation of certain Fehling reducing sugars. For instance, a Fehling-reducing sugar which is gas-fermented by *B. typhosus* + *Morgani* and not by *B. kandiensis* + *Morgani* is probably maltose. The explanation is as follows: The symbiosis *Typhosus* + *Morgani* and the symbiosis *Kandiensis* + *Morgani* produce gas in the same Fehling-reducing sugars, except maltose, in which *Typhosus* + *Morgani* produces gas and *Kandiensis* + *Morgani* does not. If a Fehling-reducing sugar, therefore, is gas fermented by *Typhosus* + *Morgani* and not by *Kandiensis* + *Morgani* the inference is that it is maltose. For the identification of maltose and other common carbohydrates, however, the mycological method I devised in Ceylon, and later worked out in England with Dr. F. E. Taylor, is much simpler[1]:

TABLE II.

Showing action of the symbioses B. typhosus + B. morgani and B. kandiensis + B. morgani on the principal Fehling reducing carbohydrates.

	Glucose	Levulose	Maltose	Galactose	Lactose	Pentose
Typhosus + Morgani	G	G	G	G	0	0
Kandiensis + Morgani	G	G	0	G	0	0

Identification of maltose by means of the symbiotic fermentation phenomenon.



Recently I have found that the symbiotic phenomenon is useful in assisting in the identification of erythritol. This substance, which as well known is obtained from certain lichens, such as *Rocella tinctoria,* is a polyhedric alcohol $C_4H_6(OH)_4$ or $CH_2OH.(CHOH)_2.CH_2OH$. It is not fermented with production of gas by any organism I have tried; it is fermented however with production of gas by the symbiosis *B. kandiensis* + *B. morgani,* while it is not fermented by the symbiosis *B. typhosus* + *B. morgani.*

Of course the finding that a given substance is gas fermented by the symbiosis *B. kandiensis* + *B. morgani* and not by the symbiosis

B. kandiensis + *B. typhosus* is not sufficient to come to the conclusion that we are dealing with erythrite, but if other characteristics of erythrite are present, it may help in the identification.

The formula:

$$\begin{cases} B.\ kandiensis + B.\ morgani \ldots\ldots G \\ B.\ typhosus + B.\ morgani \ldots\ldots 0 \end{cases}$$

may indicate several other carbon compounds apart from erythrite, principally adonitol, isodulcitol, inositol. To exclude adonitol the symbiosis *B. coli* + *B. morgani* may be used; if gas is not produced it is not adonitol; to exclude isodulcitol and inositol the simple fermentation with *B. paratyphosus* B may be used; if there is no production of gas the substance is neither inositol nor isodulcitol.

This is a preliminary report.

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[1] Castellani, A., and Taylor, F. E., *J. Am. Med. Assn.*, 1926, lxxxvi, 523-527. Castellani, A., *J. of Trop. Med.*, 1926, lxxx, 217-226; *J. Am. Med. Assn.*, 1926, lxxxvii, 15-22; PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 481; *Brit. Med. J.*, 1925, ii, 734; (1904) Meetings Ceylon Branch B. M. A. (yeast).

Fiallos, J. M., *J. Trop. Med.*, 1925, xxviii, 426-428.

[2] Shütze, H.—Communication by letter.

[3] Harde, H.—Communication by letter.

3440

The use of Ionized Gas Electric Lamps as Recorders in Measuring the Velocity of the Pulse Wave.*

ROY H. TURNER. (Introduced by J. H. MUSSER.)

From the Department of Medicine, Tulane University.

Bramwell[1] and his co-workers have devised an accurate method for measuring the velocity of the pulse wave, and have made valuable studies of the factors influencing it. They have shown that the two principal factors causing increased velocity are inelasticity of the arterial walls and a high diastolic blood pressure. That is, there is an organic and a functional cause for increased velocity. The greatest need for such a method is to differentiate between the two. It seems that this might be done by observing the effect on pulse wave velocity of reduction of diastolic pressure by means of drugs. No such study has been made, due to frequent accidents with the

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* Aided by a grant from the Schwartz Research Fund.

fragile instrument used. An instrument is needed which is rugged enough to be quite dependable and yet sensitive to the highest degree.

While working in the laboratory of Dr. E. P. Carter in 1924, I devised a method, as yet unpublished, for recording arterial and venous pulsations in which the string galvanometer was used as the recording instrument. A specially designed microphone, similar to that used in telephone transmitters, was placed directly over the artery so that the pulse wave caused increased pressure on the carbon granules and thus increased the current that might pass through. Fluctuations in the current caused by the pulse were recorded by photographing the oscillations of the galvanometer string, giving an accurate picture of the pulse wave. By the use of two galvanometers focused on the same strip of film, or a galvanometer with two strings, and with two microphones placed over arteries at different and measured distances from their bifurcation, it is possible to measure accurately the velocity of the pulse. Or, by using one galvanometer for recording the electrocardiogram and the other for recording the peripheral pulsation, and by measuring from the Q-R-S complex to the up-stroke of the pulse wave, the time elapsing from the contraction of the heart muscle until the pulse reaches the microphone is determined. By the use of the two-meter roentgenogram of the heart and aorta, it is possible to measure the length of the arteries traversed in the measured interval of time, and thus determine the velocity of the pulse. However, this method requires expensive galvanometers and unless great care is used too much current may be sent through the string, causing it to either break or become non-conducting.

With the development by engineers of the General Electric Company at their Edison Lamp Works, of electric lamps capable of becoming lighted and dark again within so short a period of time as one one-millionth of a second, a new recording instrument was made available to the cardiologist. Through the kindness of Dr. L. C. Porter, of General Electric, I have had the opportunity of using this type of lamp as a marker in measuring the velocity of the pulse. These lamps depend for their source of light upon the ionization by electric potential of such a gas as neon or helium, and not upon the heating of a filament. As compared with the string galvanometer, these lamps are recorders of great ruggedness and yet quicker in response. Below a voltage of 130 the lamp is entirely dark, but if the voltage is increased it becomes lighted. A current of low voltage is passed through the microphone which is placed directly over the artery. Each pulse wave causes a corresponding pulsation in the electric current. By the use of vacuum tubes this pulsating current is so amplified that each pulsation causes a flash of a light. These flashes are recorded on the moving photographic film simultaneously with the electric cardiogram. It is not difficult to use two microphones and two lamps and record their flashes along with the electrocardiogram on the ordinary 6 cm. photographic ribbon. By the aid of the usual time marker, it is easy to determine the time interval between the contraction of the ventricle and the arrival of the pulse wave at either microphone.

It is believed that this instrument will make it possible to study accurately a large number of cases under varying conditions. It seems most likely that these lamps will be found useful as recorders in many types of cardiovascular instruments other than that just described.

A complete description of the apparatus and method, with evidence as to its limitations and possible usefulness, will be published shortly. This is a preliminary report.

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[1] Bramwell, J. C., *Quarterly J. of Med.*, 1924, xvii, 225.

3441

Studies on Immunity to Measles.

J. H. MUSSER.

From the Department of Medicine, Tulane University.

In 1925, Tunnicliff,[1] working with the diplococcus she had isolated from cases of measles, found that almost invariably a skin reaction was produced in persons who had not had measles, whereas in individuals who had had the disease, 96 per cent gave no reaction. Her antigen was a culture of organisms grown anaerobically in 1 per cent dextrose broth to which was added ascitic fluid, killed with 0.5 per cent phenol. Ferry and Fisher,[2] with an organism probably the same as Tunnicliff's, tested 35 individuals with negative measles history, and found 14 gave positive skin reactions. Thirty children who had had measles gave a negative reaction.

In the present studies, the 24-hour bouillon culture filtrate for organisms isolated by blood culture and prepared by Duval was used. Two-tenths of a cubic centimeter of a dilution of 1 to 10 was injected intracutaneously with the following results. In a home for children, with 18 children without record of having had measles, 17 gave positive reaction varying in size from 1 by 1.5 cm. to 2.5 by 5 cm. One child was negative, and on going over this child's record it was found that he had had measles. One child with a 3 by 2 reaction developed measles the day following the reading made 18 hours after the injection. Eight students with a history of measles were tested, and two gave a faint questionable reaction. One was subsequently retested with a dilution of 1 to 2 and gave a strongly positive reaction. Thirteen small children in the Charity Hospital were tested and 8 gave a positive and 5 a negative reaction. Three of the patients with a positive reaction subsequently developed measles. One of the children with a negative reaction was kept for 3 weeks in the measles ward without developing the disease. Fifteen convalescent measles cases gave a negative reaction. One child, just as rash was appearing, gave a positive reaction which two days later was negative; another a slight, faint erythmia 2 x 3 cm.

Summary. The toxic filtrate of a bouillon culture of organisms isolated by blood culture when injected intradermally into susceptible individuals causes a transient erythmia, whereas in persons who have had measles, the skin reaction is negative: Confirmatory proof that there is a specific, now isolated, organism of measles which produces a toxin that may be used as an index of immunity to the disease.

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[1] Tunnicliffe, R., *J. Inf. Dis.*, 1925, xxxviii, 193.

[2] Ferry, N. S., and Fisher, L. W., *J. Am. Med. Assn.*, 1926, lxxxvi, 932.

3442

Further Studies Upon the Etiology of Measles.

C. W. DUVAL and R. J. HIBBARD.

From the Department of Pathology of the Tulane University School of Medicine.

In a previous communication[1] we reported having obtained in pure culture a hemophilic diplococcus from the unfiltered blood of human measles, which apparently was identical to the coccus isolated by Tunnicliff[2] and similar in some respects to the coccus described later by Coronia.[3] These workers regard their respective isolation as the active exciting agent in measles. In our earlier report we reserved opinion regarding the possible causal relation that the coccus might bear to measles, inasmuch as it was obtained from the unfiltered blood, and did not conform with our conception of the excitant of measles (filterable virus).

A recent epidemic of measles in New Orleans has afforded the opportunity to make further studies concerning the incidence of this coccus in the disease.

Blood was secured by veni-puncture from 6 children in the active eruptive stage of measles, and immediately defibrinated. Within 3 hours after the blood was collected 1 cc. portions of both filtered and unfiltered was cultured upon a variety of nutrient media. At the same time rabbits were intravenously inoculated with 1 cc. quantities of the filtered and unfiltered measles blood. The purpose of the animal injections was to serve as controls upon the infectiousness of the blood employed, and also to attempt the cultivation of the coccus in question from the blood of those reacting to the inoculation.

The special nutrient medium which was found most suitable for the initial growth of the coccus was semi-solid sheep serum agar, which had been prepared in tall sterile tubes, and the reaction adjusted to approximately 0.6 per cent acid to phenothalein. The culture medium was inoculated with the measles blood by means of a long fine capillary pipette. This was introduced to the bottom of the culture tube and the content slowly ejected from the pipette as it was withdrawn through the column of medium. First generation growths of the coccus were also attempted from the measles blood by spreading 1 cc. quantities of the inoculum over the slanted surface of human blood agar. This blood agar medium was at least 1 week old before it was used for cultivation purposes, as in our experience the freshly prepared blood medium is not suitable.

In the cultures prepared from the blood of all the cases of measles studied, a small Gram positive diplococcus was recovered in pure culture upon the special media above mentioned. The cultivated coccus completely corresponds in all 6 instances, and also to that of Tunnicliff's and our own previous isolations. It is noteworthy that in no instance was there encountered in our cultures any microorganism other than the coccus described.

As pointed out by Tunnicliff, the primary culture of the coccus is obtained with considerable difficulty; however, once visible growth is established, the subplants grow readily and correspond in their general macroscopic appearance to a freshly isolated culture of *B. influenzae*. To obtain the first macroscopic colonization of the coccus we have found it necessary to incubate the primary culture at 37° C. for 10 to 14 days. At the end of this period there is seen for the first time on microscopic examination of the stained preparation a few Gram positive diplococci and short chains of definite coccal involution forms. After the second or third transfer to fresh media of a culture at this stage of development, subsequent generations grow readily in visible form under aerobic conditions at 37° C.

It appears that this coccus from the blood of measles is not only filterable but apparently invisible for that period in which it is living in an *in vivo* environment. Aside from the question of its possible etiological relation to the disease, there is no doubt that the microorganism exists in a filterable form in the circulating blood during the eruptive stage of measles. Tests which we have carried out proved that cultures of this coccus growing well on blood agar medium are not filterable through the Berkfeldt N candles. No growth occurs upon media inoculated with large amounts of the culture filtrate. Likewise rabbits inoculated with quantities of culture-filtrate fail to react, while the animals injected with the culture react leucocytically and pyrexially. Furthermore, the coccus may be cultured from the filtered blood of reacting animals, but the same difficulty is experienced in obtaining the initial artificial growth as in the instance of the human measles blood.

It is of interest to note that the coccus grown anaerobically for several generations becomes quite small and will pass through the Berkfeldt N filter. We have further determined that the coccus can be recovered directly from the host by aerobic methods of cultivation. In our hands it has not been necessary, as claimed by Tunnicliff, to employ anaerobic methods in order to obtain the primary growth upon artificial media.

The fact that we have repeatedly obtained a culture of this microorganism in the non-filterable form from the Berkfeldt N filtered infected blood is of unusual interest, since it proves for this bacterium a biological capacity to modify its size in accordance with environmental conditions.

A filterable form of a living microorganismal agent occurs regularly in the circulating blood of measles cases during the eruptive stage, and is cultivable from the Berkfeldt N filtered blood upon special media where it assumes and retains in this environment a spherical or coccal shape, measuring on the average 0.5 micron in diameter. It would seem that the presumably invisible filter-passing form is a constant feature of the microorganism in an *in vivo* habitation, while the larger visible form (non-filterable) is its basic morphology in an *in vitro* environment.

The coccus, in culture, is a facultative aerobe, and decidedly positive to Gram's method of staining. It grows commonly in pairs and short chains whose individual cells are always spherical. Macroscopic colonization of the coccus under favorable growth conditions is comparable to that of the freshly isolated *B. influenzae.*

Cultures of the coccus injected into the rabbit induces after an incubation period of 4 to 5 days a pyrexia and leucopaenia. The coccus may be again recovered in culture from the filtered blood of the reacting animal.

The bouillon culture-filtrate of the coccus (after 5 to 7 days growth to 37° C.) produces in the skin of humans susceptible to measles a characteristic reaction, and no reaction in the convalescent or person immune to measles. This phase of the work has been done by Dr. J. Musser and is reported concurrently in this issue of the PROCEEDINGS.

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[1] Hibbard, R. J., and Duval, C. W., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 853-856.

[2] Tunnicliff, R., *J. Am. Med. Assn.*, 1917, lxviii, 1028.

[3] Caronia, *Pedicatia*, 1923, xxxi, 801.

3443

Experimental Production of Carcinoma-like Epithelial Metaplasia in Mammary Gland of the Dog.*

WILLIAM H. HARRIS.

From the Department of Pathology of the Tulane University School of Medicine.

In a previous communication[1] the histological changes produced in the mammary gland of the rabbit by the inversion of nipple flaps were reported. Various proliferative changes in the inverted skin and glandular epithelium occasioned by the procedure were described. The defensive factors of the rabbit host to the continued growth of the stimulated and proliferating epithelium were noteworthy. Most of the nodules thus produced were eventually fibrosed or absorbed. Certain of the larger nodules, persisting now far more than a year, have been found to contain altered skin and its appendages which produce retention cystic masses.

As tumors of the mammary gland of the rabbit rarely occur it was concluded that even though the normal glandular epithelial cell

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* Aided by a grant from the B. M. Harrod Cancer Research Fund.

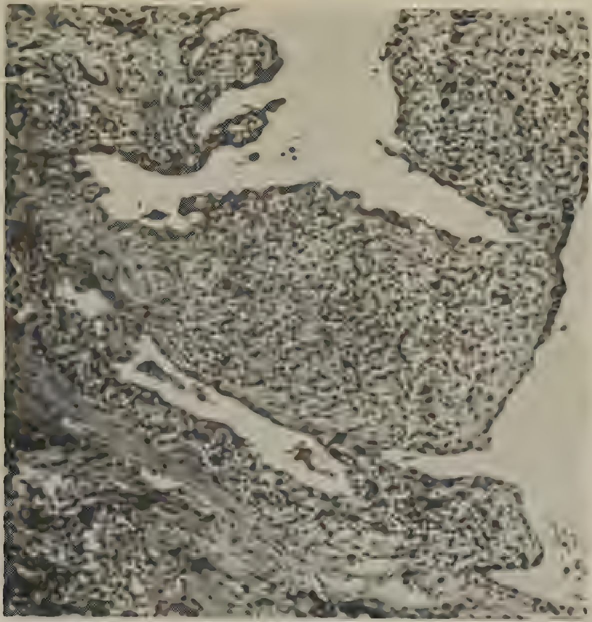

FIG. 1.

Papillary protrusions within the ducts shown after three months inversion.

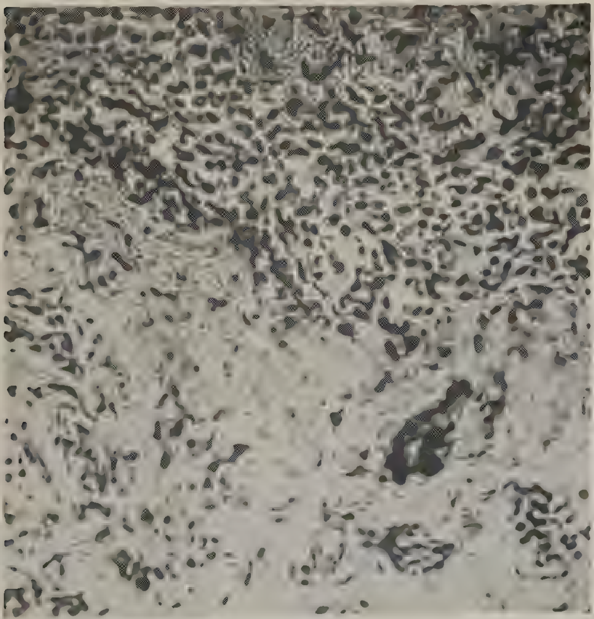

FIG. 2.

Epithelium growing at random is shown in upper portion and more or less normal ducts below.

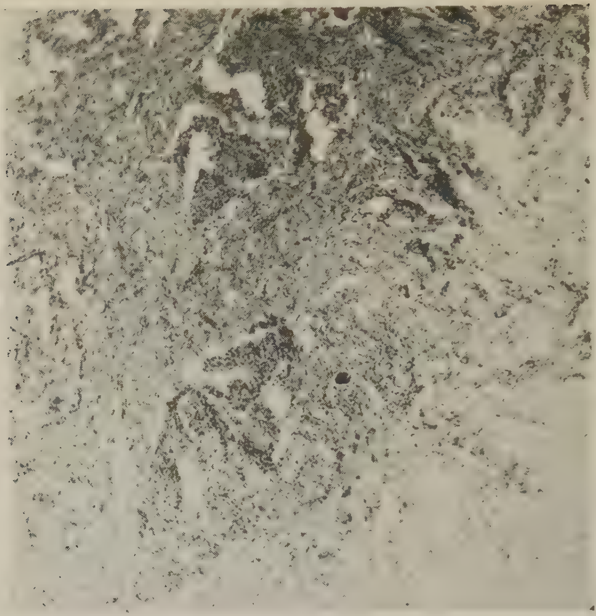

FIG. 3.
Low power to show epithelium growing throughout surrounding connective tissue.

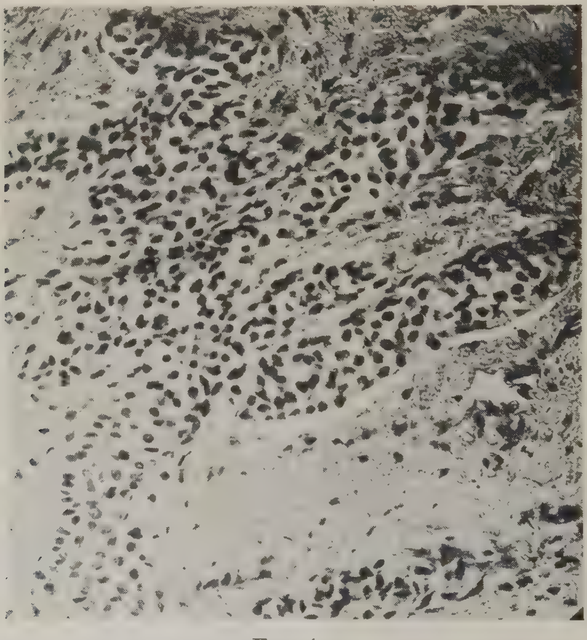

FIG. 4.
Cord-like masses of epithelium which have broken through the basement membrane after six months inversion.

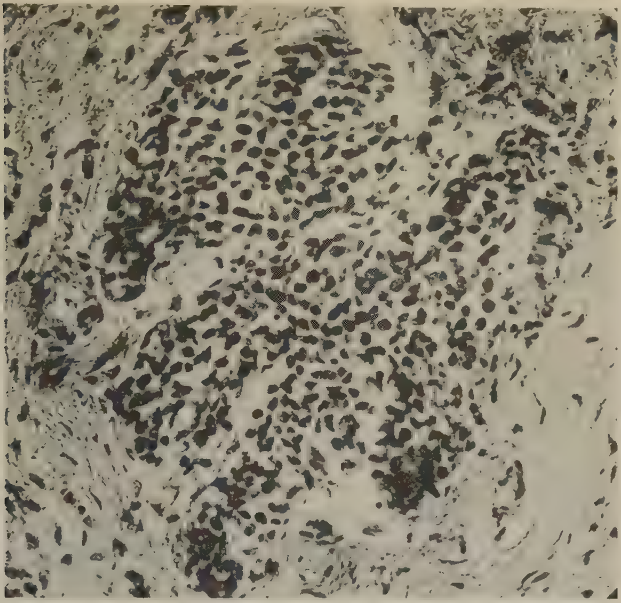

FIG. 5.

Epithelial cell masses are shown infiltrating into the adjacent connective tissue.

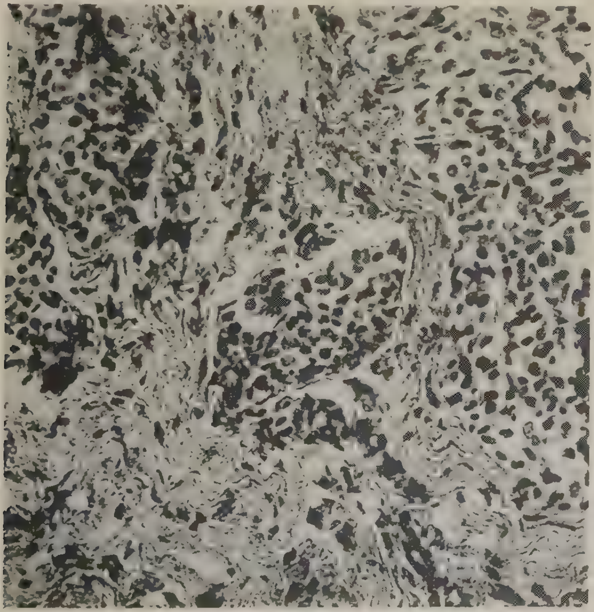

FIG. 6.

Alveolar arrangement of the epithelial cells shown in a nodule after seven months inversion.

relationships had been greatly disturbed and the epithelium provoked to considerable proliferation, the lack of susceptibility of the animal to such tumor growths or rather its capable defensive barriers prevented the continued growth.

For these reasons it was considered that some other animal of sufficient size and known to be susceptible to epithelial neoplasia of the mammary gland would be preferable. The literature shows that dogs are victims of mammary carcinoma. In 603 cases compiled by Stecker[2] 299 (49.5 per cent) were mammary carcinoma. In 50 of these, where the age was known, 23 occurred between the ages of 7 and 10 years. Feldman[3] studied 132 tumors from various animals among which 17 were from dogs. Of these 17 only 3 were carcinoma, the location of which are not mentioned. Although the incidence of occurrence of mammary carcinoma in the dog is difficult to ascertain, nevertheless such an animal is at times a victim to cancerous growth of the breast.

Seven female dogs of unknown age have thus far been employed. One of these showed slight lactation. They could only be judged as old where certain appearances such as worn teeth, advanced mammary gland development and the like were present. The age is an important feature, as the cancerous period in the dog like in the human, is noted especially during certain ages.

The procedure in general varied from the technique employed in the rabbit in that no inversion of the skin, as part of the flap, was made. An elliptical incision was carried around the nipple and the skin proximal to the nipple from this incision was removed. The portion of modified skin immediately over the nipple was clipped and scarified. The skin at the edge of the denuded surface was then dissected back for about 3 cms. thus leaving a protruding apex nearly devoid of epithelium. An incision penetrating the breast was made which extended to the underlying musculature. A suture was passed externally to the detached skin through to the underlying muscle at the base of the gland incision. The nipple was then threaded with this same suture, and returned along its path of entrance, and when drawn by both ends, detracted the nipple flap down to the musculature. The surface structures were then approximated with linen sutures, thus completely covering the operative area.

This procedure provoked a variable amount of inflammatory reaction with, at time, some suppuration, however no necrosis of the flap occurred. After from 3 to 7 months when all inflammatory signs had disappeared, portions of the persistent nodules were removed for examination. In all instances the mammary ducts showed marked epithelial proliferations within the lumen, forming at times papillary protrusions. In the majority of nodules certain of the areas present the histological appearance of carcinoma, the epithelium proliferating beyond all normal confines of limiting basement membrane. These nodules thus far have become quiescent in their growth, and no evidence of metastasis to adjacent lymph nodes have as yet been observed. The histological picture of carcinoma is not general throughout the nodules, but only appears in certain areas. Thus while the histologic study reveals cell arrangements like those of carcinoma, the general growth activity and propensity of metastasis have thus far not been demonstrable. In other words the general character of the nodule is as yet analogous to the various "cell rests" that occur in the human body.

In the experiments herewith reported[1], histologic pictures like those of cancer have been produced by causing deranged epithelial cell relationship through nipple and gland inversion although its nodules have thus far not demonstrated the usual functional activities of cancer. It is to be noted, in this connection, that cancer of the breast of the dog, is as a whole, a less active process than that of the human and instances of fibrosis and metaplasia of these growths are reported.

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[1] Harris, W. H., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 840.

[2] Stecker, cited by Müller and Glass, Diseases of the Dog, 5th ed., 1926, 339.

[3] Feldman, W. H., *Am. J. Path.*, 1926, ii, 545.

Minnesota Branch.

University of Minnesota, February 23, 1927.

3444

Induced Large Variations in the Urinary Proteins in Bright's Disease, Particularly in Nephrosis.

HILDING BERGLUND AND GRACE MEDES.

From the Medical Service of the University Hospital, Minneapolis.

In a previous report[1] some of the factors influencing the albumin output through diseased kidneys in chronic nephrosis and in animal experiments were discussed. The present work is a continuation of this investigation, using three patients with chronic nephrosis.

With two of the patients the same sudden rise under high protein diet (130 gm. daily) occurred as in the previous experiments, with immediate fall to the original level upon return to a low protein diet (30 gm. daily). The globulin to albumin ratio in the urine remained approximately constant.

During a period of fever (body temperature about 38.5°) of one of the patients, the urinary proteins increased to a still higher level, and the globulin to albumin ratio also increased, indicating a greater permeability of the kidney. That the kidney was probably damaged was indicated by the slow return of the urinary proteins to their former level, as contrasted with the sudden fall after high protein feeding.

The third patient was a case of myxedema combined with nephrosis and showed a metabolic rate of —30 at the beginning of the experiment. The induced increase of urinary protein was insignificant as compared with the results in other cases. After a short period on high protein intake, the patient was given thyroid extract daily, whereupon there occurred an immediate large rise in the protein output followed by a gradual decline during the ensuing period. This sudden increased output of protein is interpreted as a confirmation of the theory of Boothby[2] that the body contains part of its protein in the form of storage protein, which, in non-nephritic cases of myxedema is being mobilized by thyroid medication and eliminated as urinary non-protein nitrogen. In the case studied by us, the whole extra nitrogen output following the thyroid medication was in the form of urinary protein.

The urinary protein in nephrosis consists of at least two proteins of marked difference in solubility. The so-called globulin fraction is chiefly precipitated between concentrations of Na_2SO_4 of 1.0 *M* and 1.25 *M*. The so-called albumin fraction, which always is much larger than the globulin fraction, shows a higher solubility than the serum albumin of the blood plasma, practically nothing coming down before a Na_2SO_4 concentration of 1.7 *M*. Comparisons between the globulin concentrations in plasma and urine as well as between the albumin in plasma and urine are therefore futile.

The large variation in urinary proteins induced in several successive cases of chronic nephrosis by sufficiently large variations of the protein intake is looked upon as far as the kidney is concerned as a passive process, not indicative of any variation in the degree of the existing kidney damage.

The excess protein coming out in the urine on a high protein feeding was finally discussed in its relation to protein digestion, amino acid absorption and protein synthesis and the hypophysis was advanced that the urinary protein under discussion represents an escape through the leaking kidney of new protein formed in the organs of the highest amino acid concentration during the post absorptive period,[3] this new protein being on its way to other parts of the body. The possibility of such a more or less unfinished protein is suggested by the high solubility found by the so-called albumin fraction of the urine. Purification of this protein is under way.

This is a preliminary report.

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[1] Berglund, H., Scriver, W., and Medes, Grace, *Proc. 18th Annual Meeting Am. Soc. for Clin. Invest.*, p. 612.

[2] Boothby, Walter, and co-workers (personal communication).

[3] Van Slyke, D. D., *Harvey Lectures*, 1915-16, xi, 146.

3445

Correlation of Loaf Volume with Peptizing Action of Salts on Wheat Flour Proteins.*

ROSS AIKEN GORTNER.

From the Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station.

We have recently completed in this laboratory an extensive study of the peptizing action of certain salt solutions on a series of wheat flours.† The 12 wheat flours used were selected with the object of including flours from the various types of wheat grown extensively in the United States and Canada. The experimental procedure was to weigh out 6 grams of flour into a 100 cc. centrifuge tube, and to add 50 cc. of a salt solution of given concentration. This mixture was then shaken for 30 minutes in a mechanical shaker, and the flour residue tightly packed in the bottom of the tube by an electric centrifuge. The clear supernatant liquid was decanted into a Kjeldahl flask and the extraction repeated on the flour residue with 50 cc. of fresh salt solution, repeating the shaking, centrifuging and decanting, and following this with a third extraction of the residue, with a fresh portion of 50 cc. of the salt solution. Sulfuric acid was then added to these combined extracts, and the nitrogen dissolved by the salt solution determined by the usual Kjeldahl-Gunning procedure. From the values thus obtained, the percentage of the wheat flour proteins soluble in the salt solution was calculated.

In all, 21 different inorganic salts were used, nearly all in four different concentrations, so that we have data as to the percentage of protein dissolved by the different salts, and the effect of concentration of the salt on protein peptization. Obviously such data are too extensive for summarization here. Great variations in the peptizing action of the various salts were observed. For example, normal solutions of KF dispersed an average of 13.07 per cent of the total protein, whereas a similar concentration of KCl extracted an average of 22.77 per cent. KBr extracted an average of 37.22 per cent and KI extracted an average of 63.89 per cent. All of these solutions had approximately the same hydrogen ion concentration, about pH = 5.87 to 6.05.

The different salts showed extreme ranges in the percentage of

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* Published with the approval of the Director, as Paper No. 679, Journal Series, Minnesota Agricultural Experiment Station.

† Unpublished data of R. A. Gortner, W. F. Hoffman, and W. B. Sinclair.

protein extracted from a single flour (*e. g.*, with flour No. 1, N/1 KF extracted 18.0 per cent of the total protein, whereas N/1 KI extracted 73.9 per cent.) There was an equally surprising variability in the percentage of total protein extracted by a single salt solution from the various flours (*e. g.*, the N/1 KF values ranged from 10.5 per cent to 18.0 per cent, the N/1 KCl values ranged from 18.7 per cent to 30.4 per cent, the KBr values ranged from 30.1 per cent to 48.6 per cent, and the KI values ranged from 55.4 per cent to 73.9 per cent). When the percentages of protein extracted by all the 21 salts (N/1) were averaged, the range between the flours was from 23.45 per cent to 37.71 per cent.

These characteristic variations in the behavior of the various flours toward salt solutions suggested that perhaps the peptizing action of the salt solutions on the wheat flour protein might be correlated with loaf volume. Accordingly the correlation coefficient between loaf volume and the percentage of protein extracted by a salt solution was calculated, using the correlation formula suggested by Harris.[1]

Thirteen correlation coefficients have been computed. The lowest correlation so far obtained (the N/1 KI series) is $r = -0.672 \pm 0.107$, and the highest (the N/1 $MgSO_4$ series) is $r = -0.925 \pm 0.028$.

These high negative correlations indicate that the larger the loaf volume, the smaller will be the percentage of protein peptized by a given salt solution. This is in exact agreement with a statement which I made[2] in 1918, to the effect that flours characterized by a small loaf volume yielded glutens which were more easily peptized than were the glutens from flours yielding large loaf volumes.

All of our studies[3] have indicated that the weak glutens were charterized by greater ease of peptization than were the strong glutens, but the present series of experiments is the only series where we have used statistical methods. There are sufficient data in one previous paper,[4] however, to justify the application of the correlation formula. Accordingly I have calculated the correlation coefficient between loaf volume and per cent of total protein soluble in 5 per cent K_2SO_4 solution for that series of flours, and find $r = -0.824 \pm 0.065$, so that the high negative correlation which we find in the present series of flours is substantiated by the data of this earlier series.

We are now preparing to begin an extensive experiment to test out the validity of the correlations on a much larger series of commercial wheat flours.

The implications involved, should our present high negative correlations be confirmed by the more extensive experiment, include (a) the possibility of calculating by means of a formula the volume of the loaf of bread which can be secured from a given sample of wheat flour; (b) the possibility of supplementing the milling and baking tests for the determination of wheat quality with a much simpler test involving the use of much smaller samples of wheat, this would be of extreme importance in plant breeding studies; and (c) the possibility of radically changing present systems of wheat grading.

Nothing can be said with certainty in regard to the above possibilities until we have completed more extensive experiments. Our present data, however, indicate that all of the flours included in the present or the previous[4] studies contained sufficient good protein to make a desirable loaf volume, but that associated with this good protein there was a certain amount of inferior, easily peptized, protein which prevented the good protein from exerting its maximum influence. Likewise the possibility of applying this method to the problems of frosted, rusted, sprouted, or otherwise damaged wheat must be obvious. (The complete data of these experiments will be published in *Cereal Chemistry.*)

We are at present engaged in another series of experiments in which we are studying the behavior of various pure proteins, some from wheat endosperm, others from other plant or from animal sources, toward the peptizing action of certain neutral salt solutions, and have already secured very striking results. Certain proteins which have hitherto been regarded as distinct entities have already been separated into fractions which show widely different properties. These data, however, are not sufficiently complete for a detailed report at the present time. This is a preliminary report.

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[1] Harris, J. A., *Am. Nat.*, 1910, xliv, 693.

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3446

A Physiologic Consideration of the Sphincter of the Ductus Choledochus.

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The presence of a sphincteric mechanism at the duodenal end of the common bile duct, in relation to the functioning of the biliary tract, has never been conclusively proved. More recently, in the light of newer studies upon the physiology of the gall bladder, it has been questioned. The muscle tone of the duodenum, together with the peristaltic waves of the gastro-intestinal tract have again been ascribed as essential factors in the flow of bile.

To determine whether or not there was a localized mechanism at the duodenal end of the common duct capable, upon stimulation, of stopping the flow of bile, the following series of experiments were performed. Under ether anesthesia, the biliary tract of a dog was exposed and a cannula inserted into the common duct with the point directed towards the duodenum; and the lower intra-mural portion of the duct was carefully dissected away as closely as possible from the wall of the duodenum. All tissue as nearly as could be determined with the naked eye, not of the duct itself, was carefully teased away. The cannula was then connected to a pressure bottle containing Ringer's solution, kept at a constant body temperature; while the intra-mural portion of the duct was carefully placed upon the points of an electrode. In this position Ringer's solution was allowed to pass through the duct and records were made, by both the drop and the pressure method, of the rate of flow of the fluid through the duct before, during, and after stimulation. A brief electrical stimulation applied to a restricted region of the intra-mural portion caused a stoppage of the flow of the fluid through the duct, at pressures greater than the recorded pressure produced by the secretion of the liver, or the contraction of the gall bladder. Following the period of stimulation, there is an interval of a few seconds before the fluid again passes through the duct, and several seconds before the normal rate is restored. A similar stimulation of any other extra-mural portion of the duct had no appreciable effect upon the flow of fluid through it. Similar studies upon the guinea pig, wherein the common duct opens directly into the duodenum, reveals the existence of a strong sphincteric mechanism at the lower end of the choledochus, wholly apart from the gastro-intestinal tract.

This is a preliminary report.

3447

The Application of Certain Statistical Criteria to the Problem of Seedling Mortality.

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The practically important problem of obtaining an adequate crop stand on the agricultural field must depend for its solution on the detailed investigation of a number of factors, physical, chemical, physiological and pathological. While the end purpose of such investigations may be in part practical, they have their bearing upon a number of general biological questions, for example that of the existence of a selective death rate.[1] In suggesting the applicability of certain statistical methods, which, as far as I am aware, have not heretofore been applied to these problems, I have in mind the probability that they will be useful in other investigations involving the problem of mortality.

Problem 1. A criterion of the deviation from a random distribution of an observed distribution of number of seedlings produced.

Let s be the number of seeds planted, g be the number of seeds germinating or surviving to a given stage, f the number of seeds failing to germinate or dying at an early stage, in a large number, N, of small experiments. Then if p be the probability of development of the seed into a seedling, and q be the probability of its failing to develop or of its dying before any given period

$$p = \frac{\Sigma(g)}{sN} = \frac{\overline{g}}{s}, \quad q = \frac{\Sigma(f)}{sN} = \frac{\overline{f}}{s} = 1 - p,$$

where Σ denotes summation throughout the number, N, of experimental plantings and the bars denote means of g and f.

If the frequency distribution of the number of germinations per experiment (*e. g.*, per hill) were determined solely by chance it should should be given by $N\ (p+q)^s$. The agreement of the theoretical and empirical distributions may be tested by Pearson's χ^2 criterion,[2] using Elderton's[3] table for testing goodness of fit.

Application of this criterion to large series of germination records for Sea Island, Egyptian and Upland cotton[4] has shown by the large values of χ^2 that in this specific case the chances of the deviations of the observed from the theoretical distributions being due to random sampling are, practically speaking, infinitesimal. These results indicate clearly the influence of some factor or factors which tends to bring about greater differences in the number of seedlings germinating per experimental unit than would be expected on the basis of chance alone. These factors may be most logically sought in the influence of substratum heterogeneity, which has been considered in a number of earlier investigations on soil properties and plant characteristics.[5]

It is here suggested that this χ^2 test furnishes a valuable criterion for the selection of the most suitable planting technique for experimental work.

Problem 2. The correlation between the mortality of associated seedlings.

In dealing with the problem of the influence of localized conditions on seed germination the method of intra-class or inter-class correlation[6] may be utilized by employing the number of seedlings per unit as variates when more than one unit can be considered to be associated. In cases in which it is desired to express the relationship between the fate of the seedlings of the same unit on a correlation scale, the frequency distribution may be reduced to a four fold table of alternative variables as follows:

Let n be the number of seeds germinating, m the number failing to germinate per experimental unit of $n + m = s$ seeds per unit. The contributions of any such unit to the four fold table will be $gg = n(n-1)$, $gf = nm$, $fg = mn$, $ff = m(m-1)$. The summation of these values for all the experimental units gives all frequencies required for the four fold table. Such tables permit the application of Pearson's equal probability r.[7]

An application of this criterion to large series of data for the germination of cotton on highly saline soils of the Gila River Valley at Sacaton, Arizona,[8] results in correlations ranging from .3635 to .7463.

Problem 3. A criterion of the differentiation of varieties with respect to germination rate, or of environmental conditions with respect to their capacity for influencing germination rate. A determination of the significance of the differentiation of two varieties with respect to germination rate or of two sets of conditions with respect to their capacity for influencing germination rate may be made by means of Pearson's[9] double χ^2 test.

Unpublished studies on cotton germination rates indicate wide variations in the extent of differentiation with respect to capacity for the production of seedling stand.

Such a method of comparison as that here suggested is desirable since it is possible that criteria of differentiation based on means and standard deviations alone may be inadequate in view of the fact that the form of the distribution may differ in the two varieties or environmental conditions compared.

This is a preliminary report.

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3448

Decorticate Rigidity.

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On section of the brain stem at the level of the colliculi, Sherrington[1] in 1897 observed that the animals (cats) passed into a condition of increased extensor tonus. This condition he called "decerebrate rigidity". The rigidity was "confined to those muscles which maintain the animal in erect attitude".

Goltz,[2] Holmes,[3] Rothman[4] and de Barenne[5] studied dogs in which the frontal portion of the brain was removed, and in which most of the thalamus and midbrain were left intact, but reported no rigidity. Thiele[6] and Magnus[7] have stated that it is necessary to section the brain in the mesencephalic region to obtain rigidity. Hence it has been generally accepted that the condition results, not from removal of the cerebral cortex, but from transection in the region of the midbrain, at or near a supposed rigidity center. However, we find that rigidity follows at once if a sufficient amount of the cortex alone is removed, leaving all other structures intact.

In our experiments four monkeys (Javanese macaques), and three dogs have been used. After removal of the calvarium, as much of the cortex was clipped off with scissors as could be reached without injury to other portions of the brain. The plane of removal was sufficiently superficial so that the portions of cortex surrounding the deep sulci were left intact. The areas of removal were in general as follows: dorsal, medial and lateral surfaces and the anterior tips of the frontal cortices, all of the parietal cortices, the posterior portion of the temporal lobes, and all of the occipital cortices, except that in contact with the anterior half of the tentorium. In every case a well marked generalized muscular rigidity appeared during recovery from the anesthesia, and the nervous responses retained were apparently entirely automatic.

The rigidity in the monkeys differed from that observed in the dogs. The monkeys showed flexor rigidity of the neck, trunk and extremities. But upon skin stimulation with a needle point they would frequently go into extensor rigidity and then slowly return to flexor rigidity. After several hours the upper or lower extremities frequently became flaccid, but would return to flexor or extensor rigidity on disturbing the animal. Similar observations have been recorded by Bazett and Penfield[8] in studying chronic decerebrate cats. The monkeys assumed various postural patterns as reported by Magnus in the study of decerebrate rabbits: when their heads were turned to the right their lower limbs went from flexor to extensor rigidity, the right side being more rigid than the left; and when their heads were turned to the left a similar picture was obtained but the left side showed more rigidity than the right. Unilateral decortication on one of the four monkeys resulted in homolateral rigidity with preservation of voluntary movements and contralateral paralysis with less rigidity than on the homolateral side.

The dogs went into extensor rigidity after decortication. When placed on the right side the extremities of the right side were most rigid, and when placed on the left side the extremities of the left side were most rigid. As in the case with monkeys, when the head was turned to the left, the extremities on that side became more rigid, and when the head was turned to the right the extremities on the right became more rigid. When placed in a sitting position with the limbs flexed, the animals developed flexor rigidity. This flexor rigidity persisted when the dogs were tipped from the sitting position on to their backs but the rigidity became extensor when the animals were placed again on their sides. Unilateral decortication was performed on one dog, and the same phenomena with homolateral rigidity and contralateral paralysis were observed as in the case of the monkey already mentioned. In one dog the usual mesencephalic decerebration was performed 2 hours following decortication. The rigidity became more marked but showed no definite qualitative changes. All the animals showed pupillary reflexes. They all shivered at times.

Karplus and Kreidl[9] extirpated both hemispheres of monkeys leaving the thalamus intact, and report tonic extremities in some of the animals. Olmsted and Logan[10] removed part of the motor cortex in cats and observed some extensor rigidity. They also observed extensor rigidity upon injury to the frontal lobes.

The foregoing observations demonstrate that removal of sufficient cerebral cortex results in rigidity, similar if not identical to decerebrate rigidity obtained by the usual midbrain section. It appears to us, therefore, that decerebrate rigidity may well be explained simply on the assumption of removal of cortical inhibition, rather than on the basis of special tonus mechanisms, which is sometimes postulated in the literature of this subject.

This is a preliminary report.

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3449

On the Carbohydrate Metabolism of Adrenalectomized Rats.

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It is known that adrenalectomized cats and dogs frequently have a blood sugar concentration below normal. These two species survive the operation only a short time, and the animals generally refuse to eat some time before death. It was, therefore, not certain whether the low blood sugar was a symptom of adrenal insufficiency, or whether it was due to the imminent death of the animals, since a low blood sugar before death from other causes is not uncommon. On the other hand, a larger percentage of adrenalectomized rats survive the operation for a long time. About 20 per cent of our operated animals 10 to 14 days after removal of both glands had maintained their original body weight or even surpassed it. Incidental to other work, it was noted that these rats showed a low blood sugar when they were fasted for 24 hours. Six rats showed a blood sugar ranging from 53 to 79 mg. per cent. A simultaneous analysis of the liver and the rest of the body tissues revealed that there was practically no liver glycogen present, while the rest of the body tissues, chiefly the muscles, showed a normal glycogen content. The absence of liver glycogen explains the low blood sugar of these animals, since the liver glycogen is the only source for blood sugar. There is good evidence that the muscle glycogen does not participate in blood sugar regulation. When normal rats are fasted for 24 hours, the blood sugar rarely falls below 0.1 per cent and there is from 0.4 to 1.0 per cent liver glycogen present. Even after a 48 hour fast there is sufficient liver glycogen present to maintain a normal blood sugar concentration. The lack of liver glycogen in fasting adrenalectomized rats may have two causes. Either the polimerisation process or the new formation of glucose from proteins is disturbed. The first possibility has been tested experimentally by determining the rate of glycogen formation in the liver after feeding glucose by stomach tube. Five adrenalectomized rats, fasted previously for 24 hours, formed an average of 3.2 per cent liver glycogen during 4 hours of glucose absorption, while normal rats formed 3.7 per cent. Glycogen synthesis is, therefore, not disturbed in adrenalectomized rats. For this reason the blood sugar and the liver glycogen of non-fasting adrenalectomized rats are normal. The second possibility has not been tested experimentally so far. The question is whether the lack of liver glycogen during fasting has to be linked with the loss of cortical tissue, or with the absence of epinephrine secretion. It is of interest in this connection that small doses of epinephrine given to animals rendered free from liver glycogen lead to glycogen synthesis (Pollack,[1] Kuriyama[2]). While large doses of epinephrine produce glycogenolysis, small doeses have the opposite effect.

When fasting adrenalectomized rats received an insulin injection simultaneously with the glucose feeding, an average of 0.9 per cent liver glycogen was formed. This inhibition of glycogen synthesis in the liver following an insulin injection has been observed previously on normal rats.[3] In experiments where 90 per cent of the absorbed glucose could be accounted for by oxidation plus glycogen formation in the liver, and in the rest of the body tissues, the amount of glucose that failed to be deposited in the liver of insulinized rats appeared in the balance as oxidized. The interpretation was that due to an increased oxidation of glucose in the muscles, as the result of a surplus of insulin, not enough glucose was left for glycogen formation in the liver. Insulin effected a shift in the disposal of glucose from the liver into the muscles. In other words, the inhibition of glycogen formation in the liver was assumed to be a secondary phenomenon. On the other hand, the hypoglycemia produced by insulin seems to lead to an increased discharge of epinephrine. (Cannon, McIver and Bliss.[4]) It was, therefore, of importance for the interpretation of the mechanism of insulin action to repeat these experiments on adrenalectomized animals. There was no marked difference in the carbohydrate metabolism of normal and adrenalectomized animals. Emphasis is here laid on the fact that the inhibition of glycogen synthesis in the liver of insulinized animals is not due to the action of epinephrine. Insulin injections have also been shown to cause a diminution of liver glycogen in animals in the post absorptive state. A repetition of these experiments on adrenalectomized mice gave the same result. This phenomenon is, therefore, not connected with an increased discharge of epinephrine.

This is a preliminary report.

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3450

Bacteriologic Studies in Acute Rheumatic Fever with Reference to Soluble Toxin Production.

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An extensive comparative study was made of the production of soluble toxins by hemolytic and non-hemolytic streptococci, isolated from a large series of normal and diseased individuals. The author encountered a strongly toxigenic strain of a non-hemolytic, non-methemoglobin-forming, inulin-fermenting, bile-insoluble, gram-positive streptococcus, isolated on March 24, 1926, from blood-cultures of a 5-year-old girl, ill with acute rheumatic fever, endocarditis, myocarditis, pericarditis, and pleurisy. Post mortem, the identical organism was isolated from the vegetations on the mitral valves, as well as from the heart's blood. In subsequent studies, a similar non-methemoglobin-forming streptococcus was regularly isolated from the tonsillar crypts, abscesses, and irregularly from blood-cultures, heart-vegetations, feces and urine, of persons with rheumatic fever and the allied forms of this protean disease. Culturally, serologically, and toxigenically, this new type of non-methemoglobin-forming streptococci was found to constitute a closely related group of micro-organisms, distinguishable from the *Streptococcus viridans* and *Streptococcus hemolyticus* groups. (Table I.) For toxin production, the tryptic medium employed was the original Douglas'[1] tryptic medium digest, modified by Hartley,[2] Watson and Wallace,[3] and the cultures were incubated at 37°

TABLE I.

Biological reactions of non-hemolytic streptococci isolated from patients with history of rheumatic fever.

Strain	Source	Glucose	Lactose	Sucrose	Mannite	Salicin	Raffinose	Inulin	Bile	Toxin	RF. -AT neutral	Agglutination Reactions		
												RF. I Serum	Str. virid. Serum	
													"SV 76"	R. 37
*Beattie—227	Blood	A	A	A	A	A	—	—	—	—	—	—	—	—
*Allen—304	Blood	A	A	A	A	A	—	—	—	—	—	—	—	—
*Swift—A. 49	Blood	A	A	A	—	—	—	—	—	—	—	—	—	1:40
*Swift—B. 38	Blood	A	A	A	—	A	A	A	—	—	—	1:80	—	—
*Swift—38 D.	Blood	A	A	A	—	—	—	—	—	—	—	—	—	—
*Swift—A.179	Heart	A	A	A	—	—	—	—	—	—	—	—	—	1:160
*Swift—W. 72	Nodule	A	A	A	—	—	A	—	—	—	—	1:40	—	—
RF. 1—b.	Blood	A	A	A	—	A	A	A	—	+	+	1:1,280	1:20	1:40
RF. 1—v.	Heart	A	A	A	—	A	A	A	—	+	+	1:2,560	1:20	1:20
RF. 2	Tonsil	A	A	A	—	A	A	A	—	+	+	1:640	—	—
RF. 3—t.	Tonsil	A	A	A	—	A	—	A	—	+	±	1:160	—	—
RF. 3—f.	Feces	A	A	A	—	A	A	A	—	+	+	1:320	1:20	1:40
RF. 4	Tonsil	A	A	A	—	A	A	A	—	±	±	1:40	1:20	1:20
RF. 5	Chorea	A	A	A	—	A	—	A	—	+	+	1:80	—	—
RF. 6	Chorea	A	A	A	—	A	—	A	—	+	+	—	—	1:40
RF. 7	Tonsil	A	A	A	—	A	—	A	—	—	—	—	1:40	—
RF. 8	Tonsil	A	A	A	—	A	A	A	—	+	+	1:80	1:20	—
RF. 9—t.	Tonsil	A	A	A	—	A	—	A	—	+	+	1:160	—	—
RF. 9—f.	Feces	A	A	A	—	A	—	A	—	+	+	1:80	—	1:160
RF. 10—b.	Blood	A	A	A	—	A	A	A	—	+	+	—	—	—
RF. 10—t.	Tonsil	A	A	A	—	A	A	A	—	+	+	1:320	—	—
RF. 10—a.	Abscess	A	A	A	—	A	—	A	—	+	+	1:1,280	1:20	1:40
*RF. 11	Blood	A	A	A	—	—	—	—	—	—	—	—	—	—
RF. 12	Tonsil	A	A	A	—	—	—	A	—	±	±	1:40	1:20	—
RF. 13—a.	Abscess	A	A	A	—	A	—	A	—	+	+	1:640	1:40	1:80
RF. 13—t.	Tonsil	A	A	A	—	A	—	A	—	+	+	1:320	1:40	—
RF. 14	Tonsil	A	A	A	—	—	—	A	—	+	+	1:40	1:20	—
RF. 15	Tonsil	A	A	A	—	A	A	A	—	+	+	—	—	—
RF. 17	Chorea	A	A	A	—	A	—	A	—	+	+	1:640	1:40	1:20
RF. 18	Abscess	A	A	A	—	A	A	A	—	+	+	1:1,280	1:40	—
RF. 21	Tonsil	A	A	A	A	—	—	A	—	±	±	1:320	—	—
RF. 24—t.	Tonsil	A	A	A	—	A	—	A	—	+	+	1:80	—	—
RF. 24—u.	Urine	A	A	A	—	A	—	A	—	—	—	1:160	—	1:160
RF. 36	Tonsil	A	A	A	—	A	A	A	—	+	+	1:1.280	1:80	—
*R 37	Blood	A	A	A	—	—	—	—	—	—	—	1:20	1:20	1:2,560
*SV. 76	Blood (SBE)	A	A	A	—	—	—	—	—	—	—	1:20	1:2560	1:40

*Typical strains of *Streptococcus viridans* type: remaining strains are non-methemoglobin-forming streptococci. T-AT neutral indicates that 0.01 of the RF. I antitoxin (rabbit) is mixed with 0.1 cc. of a 1 to 100 dilution of the toxic filtrate and is injected intradermally. + indicates complete neutralization: ± partial neutralization, and — no neutralization. A indicates acid fermentation without gas-production. SBE implies subacute bacterial endocarditis.

C. over periods varying from 4 to 10 days, when the cultures were filtered through Berkefeld V candles. Ninety-eight strains of hemolytic streptococci and 247 strains of the green streptococci yielded only 4.7 per cent of toxin producing strains. Among 68 strains of the non-methemoglobin-forming streptococcus isolated from rheumatic fever, 49 strains, or 72 per cent were found to produce a demonstrable soluble toxic filtrate, slightly weaker in toxicity than that obtainable from *Streptococcus erysipelatis*.[4] When normal persons without history of rheumatic fever were tested intradermally with a skin test dose of 0.1 cc. of 1 to 100 dilution of the soluble toxic filtrate produced by the non-methemoglobin-forming streptococcus, isolated from rheumatic fever, 18 per cent of adults and 11 per cent of children gave positive skin reactions, similar to the Dick test, and measuring more than 1 cm. in diameter within 24 hours after the injection. Among persons with history of rheumatic fever and tested with 0.1 cc. of 1 to 100 dilution of this soluble toxin, 56 per cent of adults and 76 per cent of children gave positive skin reactions within 24 hours after the injection. When the same persons were tested with a lower dilution of 0.1 cc. of 1 to 10, it was found that 67 per cent of adults and 85 per cent of children gave positive reactions within 12 hours following the intradermal injection. In many instances the local swelling simulated erythema nodosum. Complete neutralization of the toxin was obtained by mixing the skin test dose with 0.01 cc. of the serum drawn from rabbits actively immunized with the toxin or with the serum drawn from individuals actively immunized by intramuscular injections of toxin-antitoxin mixtures.

Thermal inactivation of the toxin produced by the non-methemoglobin-forming streptococcus isolated from rheumatic fever, was first detected after boiling the filtrate for 1 hour. Exposing the toxin to direct sunlight and room temperature for more than 3 months failed to destroy the toxic principle. The active toxic substance was completely removed with the fraction precipitated by 6 volumes of absolute alcohol. The resultant white and flaky precipitate was readily redissolved in normal saline solution and in this manner could be purified and concentrated with great ease.

Intravenous and subcutaneous injections in the rabbit of the living non-methemoglobin-forming streptococcus, invariably produced a non-suppurative polyarthritis, with marked swelling of the joints. The joint fluid was mucoid in appearance, containing numerous polymorphonuclear cells and no bacteria. Subacute bacterial endocarditis and myocarditis, with a unique and regular tendency to vegetative mitral stenosis, extensive mural vegetations, and occasionally pseudo-nodular arrangements of multinucleated forms of leucocytes, as well as of polymorphonuclear and mononuclear cells, were encountered. Intravenous and subcutaneous injections of the sterile toxic filtrate rapidly developed polyarthritis of the non-suppurative form, after repeated injections of increasing doses. The only changes found in the heart of the rabbits injected with the sterile toxic filtrate, were those of myocardial degeneration.

After seven months of constant intradermal injections of skin test doses of the sterile toxic filtrate of the non-methemoglobin-forming streptococcus, in which time the positive skin reaction remained unchanged in degree of cellular response, the author injected himself intra-articularly in the left wrist and intramuscularly in the right forearm with 1 cc. of the undiluted and sterile toxic filtrate. Twenty-four hours later general malaise developed, and chills, temperature and drenching perspiration preceded irregular joint pains. Within 48 hours after the injections, a typical clinical picture of acute polyarthritis of the rheumatic type developed, attended by hyperpyrexia, rapid pulse, excessive perspiration and migration of arthritis from one large joint to another. On the fourth day of the illness salicylates were taken in large doses and the general condition was rapidly restored to within normal limits. A secondary recrudescence of the lesions in the skin produced by the intradermal injections of the toxins occurred simultaneously with each fresh attack of arthralgia during the entire course of the migratory polyarthritis, similar to the Arthus phenomenon,[5] and the Andrewes, Derick, Swift "secondary reaction."[6] The frequency of positive reactors with the toxin produced by the rheumatic fever non-methomoglobin-forming streptococcus especially among persons stigmatized by rheumatic fever, has suggested to the author a state of allergy in such persons in relation to the toxins produced by the new species of the non-methemoglobin-forming streptococcus described in these studies and no doubt previously encountered by Mandelbaum[7] in 1907, as *Streptococcus saprophyticus*, by Zangemeister[8] in 1910, as *Streptococcus anhemolyticus vulgaris*, by Rosenow[9] in 1914, from the joint fluids in acute rheumatism, articular and muscular, and while my own studies were in progress, by Small[10] as *Streptococcus cardioarthriditis*.

This is a preliminary report.

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[9] Rosenow, E. C., *J. Inf. Dis.*, 1914, xiv, 61.

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3451

Further Observations on Apparent Effect of Diphtheria Toxin on Growth of Bacteria.

J. M. SHERMAN, C. N. STARK AND PAULINE W. STARK.

From Cornell University, Ithaca, New York.

In a previous report[1] we have noted the fact that diphtheria toxin when added to cultures of *Bacillus cereus* in a concentration of 30 m.l.d. per cc. leads to a slight but definite retardation of growth. While our data do not prove that the inhibitory factor for *B. cereus* is in fact the toxin rather than some other by-product of growth of the diphtheria organism, there is some basis for believing that this might be the case. Of particular interest is the fact that the addition of antitoxin appears to counteract this inhibitory action.

In the present paper we wish to record that similar results have been obtained with *Proteus vulgaris* and *Staphylococcus albus*. The apparent inhibitory effect of the diphtheria toxin upon the growth of these organisms is of about the same magnitude as in the case of *B. cereus*.

This is a preliminary report.

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[1] Sherman, J. M., Stark, C. N., and Stark, Pauline W., *J. Bact.*, 1927, xiii, 45.

3452

The Destruction of Botulinum Toxin by Intestinal Bacteria.

J. M. SHERMAN, C. N. STARK AND PAULINE STARK.

From Cornell University, Ithaca, New York.

In our studies of the destruction of toxins by bacteria a number of interesting, and perhaps important, observations have been made. Among these the destruction of the toxin of *Clostridium botulinum* by certain intestinal types of bacteria may be worthy of particular note.

Several investigators have shown that if large numbers of detoxified botulinum spores are fed to experimental animals death from botulism will ensue, indicating that, under some conditions, the organisms can grow and produce their toxin in the intestinal tract. Since it is well known that the organisms of botulism are frequently ingested with such foods as raw vegetables, fruits and milk, the question naturally arises as to why the disease is not commonly transmitted in this way. It may also be questioned whether some of the so-called autointoxications are in fact incipient botulism arising from the limited growth of these organisms in the intestinal tract, with the production of sublethal quantities of toxin.

It occurred to us that the probable explanation was to be found in the action of the intestinal bacteria upon the toxins which might be formed in the alimentary tract. In Table I are given data which indicate the destruction of botulinum toxin by *Bact. coli, Bact. communior, Bact. aerogenes* and *Proteus vulgaris.* In these experiments botulinum toxin (A type) was added to beef infusion broth so that the resulting medium contained approximately 100 m.l.d per cc. This toxin-broth mixture was inoculated with the different test organisms and allowed to incubate at 20° C. for two weeks. Unin-

TABLE I.

Action of bacteria upon botulinum toxin.

M. L. D. Toxin Injected	1	2	3	4
Controls	+	+		
Bact. coli	—	—	+	+
Bact. communior	—	—	+	+
Bact. aerogenes	—	—	—	+
Proteus vulgaris	—	—	—	—

+ = Death of guinea pig with typical symptoms of botulism.

— = Survival of guinea pig.

oculated tubes of the toxin-broth mixture were of course incubated at the same temperature over the same period of time and subjected to the same filtering process in order to serve as controls. The unit amounts of toxin given in Table I are in terms of approximate m.l.d. values as determined on the control tubes after incubation.

The results obtained with these organisms do not reveal a very active destruction of the toxin (not nearly so great as results we have obtained with other organisms) but sufficient, we believe, to explain the fact that the ingestion of small numbers of spores of the organism of botulism leads to no apparent ill effects.

3453

The Labile Sulfur of Insulin.

VINCENT DU VIGNEAUD. (Introduced by John R. Murlin.)

From the Department of Vital Economics, University of Rochester.

All observers agree that insulin material contains sulfur and Abel[1] has shown that sulfur is present in a very labile form, being split off as a sulfide by boiling with weak alkali. Abel believes that the sulfur is roughly proportional to the hypoglycemic potency. Brand and Sandberg[2] have shown that although the sulfur of cystine is relatively stable, the linkage of other amino acids with cystine so affects the sulfur that it is easily split out by boiling with weak alkali. Cystine can be determined quantitatively by the uric acid reagent of Folin and Denis, as shown by Folin and Looney.[3] The cystine is first reduced by sodium sulfite in saturated sodium carbonate to cysteine which then gives a deep blue color with the phosphotungstic acid reagent. Shonle and Waldo[4] found that insulin preparations respond to this test for cystine.

I have observed that insulin preparations do not give the reaction directly with the uric acid reagent but do so after reduction with sulfite. The insulin material upon boiling for thirty minutes with 0.1 N Na_2CO_3 gives a positive reaction without previous reduction, due to the formation of sulfide ion. Acid hydrolysates of the insulin material give the reaction only after reduction with sulfite. They do not, however, give the reaction directly with the uric acid reagent after boiling with 0.1 N Na_2CO_3, showing that the sulfur has somehow become more stable in the fragment split off by acid hydrolysis. It is necessary to reduce first with sodium sulfite to obtain the reaction as in the case of the unboiled acid hydrolysate.

This difference in the stability of the sulfur in the original and in the acid hydrolysed material and the behavior towards the uric acid reagent can be explained on the assumption that a S-S linkage exists in the insulin material. In the acid hydrolysate the sulfur is in a more stable form but when the fragment containing the sulfur was linked with the other groups the sulfur became labile. This is similar to the effect which the linkage of other amino acids to cystine has on the stability of sulfur in cystine. The general behavior, therefore, is very much like what would be expected of an amino acid complex containing cystine. There is, as yet, however, no proof that insulin as such contains this sulfur; for ordinary proteins like casein, gelatine, egg albumin, and Witte's peptone behave similarly to insulin in their response to the uric acid reagent under the above conditions.

The reactions mentioned above are being studied on a quantitative basis to correlate, if possible, the potency of insulin preparations with the intensity of the reactions.

This is a preliminary report.

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[2] Brand, E., and Sandberg, Marta, *J. Biol. Chem.*, 1926, lxx, 381.

[3] Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, li, 421.

[4] Shonle, H., and Waldo, J. H., *J. Biol. Chem.*, 1924, lviii, 731.

3454

Method for Study of Muscular Activity of Intestinal Segment During Perfusion Through its Lumen.

JOHN B. POLANSKY. (Introduced by John R. Murlin.)

From the Department of Vital Economics, University of Rochester.

A method for the study of the muscular activity of the intestinal segment permitting a continuous flow through its lumen of a fluid which is readily replaceable by another is presented. A segment, suspended vertically, is attached by its cephalad end to a stationary glass inlet-tube and by its caudal end to a movable glass outlet-tube. The stationary tube immediately branches into two, each branch receiving fluid from a bottle which delivers at constant pressure. In both bottles the pressure is adjusted to the same value before the actual experiment is begun, so that, when one fluid replaces the other, any change in the muscular activity of the segment cannot be ascribed to a pressure difference. The outflow may be so regulated that any desired rate of perfusion can be maintained. In the outlet system a graduated side arm is arranged in communication with a tambour to record the pressure changes within the segment. These pressure changes produce volume changes in the graduated side arm which calibrates continuously the record made by the tambour. The contractions of the longitudinal fibers are simultaneously recorded by attaching a thread to the glass outlet tube and bringing it over a system of pulleys to a lever. The segment with its attachments is immersed in a beaker of oxygenated Locke's solution kept at body temperature. The method has proved useful in the study of the influence of extracts on the muscular coats after passage through the mucosa.

This is a preliminary report.

3455

Depancreatized Dogs Kept Alive Several Months with Insulin Administered by Stomach Tube.

JOHN R. MURLIN.

From the Department of Vital Economics, University of Rochester.

Gaebler and Murlin[1] have found that insulin in ox-blood serum administered to phlorhizinized dogs by stomach tube caused positive, though small, effects on the respiratory metabolism and on the excretion of sugar, indicating combustion of more glucose than was burning just before the insulin was given. Blood serum was chosen because of its anti-tryptic action.[2] The insulin effect was not so great nor so prolonged as had been obtained earlier[3] with a salt precipitate of insulin on depancreatized dogs. Recently a number of dogs totally deprived of the pancreas and fed on meat have been kept alive for periods of six weeks to four months on daily doses of 40 to 100 clinical units of insulin in blood serum administered carefully by stomach tube in such a way as to preclude the possibility of absorption from the mouth. The effect on respiratory metabolism is immediate. Respiratory quotients as high as 1.06 have been obtained and in several instances, *as reported with salt precipitates,*[3] *the capacity to oxidize sugar has persisted for 24 hours and more.* Frank, Nothmann and Wagner[4] have observed similarly prolonged effects from the use of their synthetic compound given by mouth.

The effect on blood sugar is not so sudden as after subcutaneous or intravenous injection and several times effects on respiratory metabolism were obtained without any measurable effect on blood sugar.

Blood serum affords partial protection of insulin against the known destructive action of pepsin in the stomach and from possible destructive action of erepsin in the intestinal mucosa.

This is a preliminary report.

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[1] Gaebler, and Murlin, *J. Biol. Chem.*, 1925, lxvi, 731.

[2] Jobling, and Petersen, *J. Exp. Med.*, 1914, xix, 239.

[3] Murlin, Sutter, Allen, and Piper, *Endocr.*, 1924, viii, 331.

[4] Frank, Nothmann, and Wagner, *Klin. Woch.*, 1926, v, 2100.

Pacific Coast Branch.

University of California Hospital, February 28, 1927.

3456

Antagonism of the Pressor Action of Ephedrine by Cocaine.

FLOYD DE EDS.

From the Department of Pharmacology, Stanford University School of Medicine, California.

Certain results with excised organs and the intact circulation in this laboratory have given rise to the suspicion that ephedrine is not invariably sympathomimetic in action. That is, the responses have not always corresponded to sympathetic nerve stimulation, and have differed from those of epinephrine on the same organs or functions. On Dr. Hanzlik's suggestion, I have tested ephedrine in cocainized animals in which sensitization occurs to the pressor action of epinephrine (sympathomimetic) with a simultaneous desensitization to tyramine (musculotropic) as described recently by Tainter and Chang.[1]

The results obtained were as follows: At the end of from 10 to 15 minutes after hypodermic injection of from 6 to 30 mgm. per kilo of cocaine, the blood pressure rises from ephedrine and tyramine in a dog, a rabbit and 2 cats were not obtained, or were much less, while the rises from epinephrine were much greater than before cocaine. From 3 to 10 times the control doses (1.5 to 5 mgm. per kilo) of ephedrine gave principally a fall of blood pressure after cocaine, or in other words, a reversal of the original pressor to a depressor action. At the same time, the heart was slowed or irregular in some of the animals and there was no definite indication of a peripheral vasodilatation, as indicated by oncometric changes. Presumably, therefore, the abolition and the reversal of the pressor action were mediated through diminished functional efficiency of the heart. The striking difference from epinephrine was the complete absence of a cocaine sensitization of the pressor action of ephedrine, indicating that ephedrine, just like tyramine, is not sympathomimetic.

These results, it is believed, further establish the value of the cocainized organism as a useful biological test object for determining the sympathomimetic action of drugs. From the standpoint of the relationship of chemical composition and structure to physiological action, and so far as the pressor action under this test is concerned, ephedrine and epinephrine are not identical pharmacologically, since the mechanisms of their actions are different; and chemically, though similar, they are also different. The reversal of the pressor action of ephedrine under cocaine indicates the need of caution in the use of ephedrine in the treatment of shock and collapse, especially when these conditions occur during cocaine anesthesia, or whenever cocaine has been absorbed.

Figures 1 and 2 illustrate typical blood pressure responses to epinephrine, tyramine and ephedrine before and after cocaine in a cat.

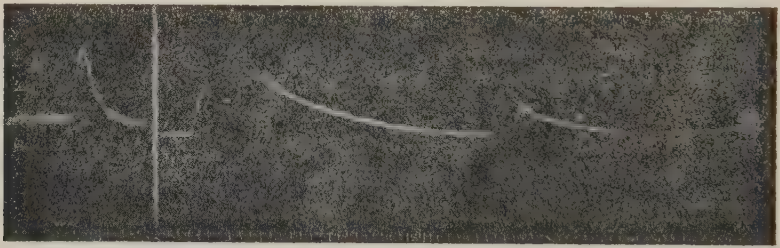

FIG. 1.

Pressor effects produced by epinephrine, tyramine and ephedrine in a urethanized cat (2.00 kg.).

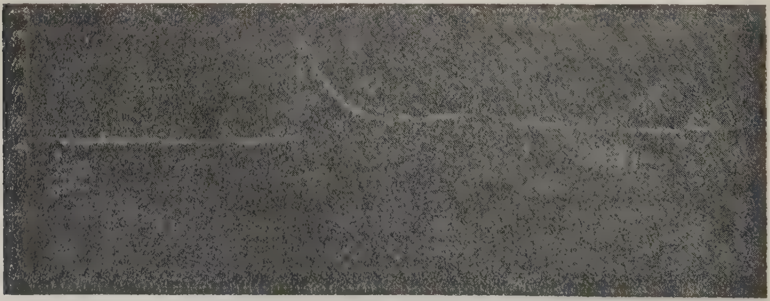

FIG. 2.

Pressor effects of ephedrine increased, and of tyramine and ephedrine abolished, by cocaine in the same cat. Records reduced to 1/3.

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[1] Tainter, M. L., and Chang, D. K., *J. Pharm. Exp. Therap.*, 1927, xxx, 193.

3457

Specificity of Hepatic Anaphylatoxins.*

W. H. MANWARING, H. D. MARINO, T. C. McCLEAVE, AND T. H. BOONE.

From the Laboratory of Bacteriology and Experimental Pathology Stanford University.

In previous papers[1, 2] it was shown (a) that normal canine organs transplanted by blood vessel anastomosis into horse serum hypersensitive dogs are thrown into typical histamine-like reactions by the toxic products (anaphylatoxins) explosively formed or liberated by the hypersensitive liver during acute anaphylactic shock in the recipient. (b) That immune organs similarly transplanted are completely resistant to the histamine-like action of these hepatic products. And (c) that this acquired anaphylatoxin insusceptibility is not due to circulating antibodies, since it can not be transferred from one animal to another by even massive immune blood transfusion. We have thought to throw light on the nature of hepatic anaphylatoxins by a study of the specificity of this acquired fixed tissue anaphylatoxin resistance.

To do this, tissue transplantations have been made between dogs immunized and sensitized with different antigens. The following is a summary of our results to date:

(1) Urinary bladders of goat serum immune dogs, transplanted into horse serum hypersensitive dogs, show no suggestion of an acquired immunity to the hepatic anaphylatoxins explosively formed or liberated during acute horse serum shock in the recipient.

(2) Horse serum immune urinary bladders, transplanted into goat serum hypersensitive dogs, show no suggestion of an acquired immunity to the hepatic anaphylatoxins explosively formed or liberated during acute goat serum shock in the recipient.

From this it is evident that the hepatic anaphylatoxins are of different chemical nature in goat serum and horse serum anaphylaxis. This finding is in accord with our working hypothesis, that hepatic anaphylatoxins are specific secondary antigens of complex protein nature, presumably denaturization products of the primary antigens used in sensitization and immunization.

This is a preliminary report.

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* Work aided by a grant from the American Committee in Scientific Research of the Medical Association.

[1] Manwaring, W. H., Hosepian, V. M., O'Neill, F. I., and Moy, H. B., *J. Immunol.*, 1925, x, 575.

[2] Manwaring, W. H., Reeves, D. L., Moy, H. B., Shumaker, P. W., and Wright, R. W., *J. Immunol.*, 1927, xiii, 63.

3458

Anaphylactic Detoxication of Specific Proteins.*

W. H. MANWARING, H. D. MARINO, T. C. McCLEAVE, T. H. BOONE, AND
E. V. ORSBORN.

From the Laboratory of Bacteriology and Experimental Pathology Stanford University.

In the preceding paper[1] we report evidence that marked chemical alterations take place in specific foreign proteins on injection into the animal body, and that certain important immunological adaptations are due to these altered or denaturized proteins, rather than to the primary proteins originally injected. We have obtained further evidence in support of this by a study of the altered anaphylactic toxicity of specific foreign proteins on intravenous injection into normal, hypersensitive and immune dogs.

Measured quantities of horse serum were injected intravenously into these animals, and at varying intervals after this injection quantitative blood transfusions were made into partially exsanguinated anaphylactic recipients. The following is a summary of our results to date:

(1) *Normal Dogs.* Quantitative transfusions into anaphylactic recipients at any time within 6 hours after intravenous injection of 2 cc. horse serum per kg. of body weight into normal dogs invariably show the circulating blood to have a greater anaphylactic toxicity than that of control amounts of unaltered horse serum. This increase suggests the possibility that the initial change in the injected horse serum in normal dogs is an increase in the number of specific protein molecules by hydrolysis or colloidal dispersion, though other explanations of this increased toxicity are of course possible.

Transfusions at the end of 24 hours show a toxicity approximately equal to that of the control horse serum dose. A slight reduction in toxicity is noted by the end of 48 hours. A toxicity equal to about a quarter of the control dose is noted at the end of 3 days. There is no recognizable anaphylactic toxicity at the end of 4 days.

Quantitative titrations with rabbit precipitin show that there is little or no reduction in the amount of circulating horse protein in the injected normal dog at the end of 4 days.

(2) *Hypersensitive Dogs.* Quantitative transfusions at any time within 4 hours after intravenous injection of 2 cc. horse serum

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* Work aided by a grant from the American Committee in Scientific Research of the Medical Association.

per kg. of body weight into hypersensitive dogs show an increased toxicity similar to that observed with normal dogs. At the end of 6 hours the toxicity is approximately that of the control horse serum dose. By the end of 10 hours the toxicity is reduced to about a quarter of the control dose. By the 15th hour the circulating blood usually becomes completely nontoxic.

Precipitin titrations show no reduction in the amount of circulating horse protein in the injected hypersensitive dog at this time.

In so far as a complete anaphylactic detoxication of horse proteins takes place by the end of 4 days in normal dogs, there is no reason to believe that the hypertrophy of this detoxicating function observed in hypersensitive dogs is necessarily due to specific antibodies.

This is a preliminary report.

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[1] Manwaring, W. H., Marino, H. D., McClure, T. C., and Boone, T. H., Proc. Soc. Exp. Biol. and Med., 1927, xxiv, 553.

3459

Toxic Substances in Friedlander's Bacillus Cultures.

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From the George Williams Hooper Foundation for Medical Research, University of California Medical School.

Two substances, toxic for rabbits, were produced in cultures of a strain of Friedlander's bacillus, originally isolated from an epidemic of pneumonia among guinea pigs.

The organism is highly pathogenic for guinea pigs and rabbits by any route of injection. Mice are also readily infected by intraperitoneal injection; a dilution of 1:10 billion of a 24-hour old broth culture (5-20 bacteria) produces the characteristic sticky mucoid exudate. Culturally, the organism forms acid but no gas in glucose, lactose, saccharose, maltose, mannite, and salicin, but not dulcite. It also reduces nitrates to nitrites, and gives a negative methyl red and Voges-Proskauer reaction.

Toxic Substance A. Procedure: The organism was grown in Huntoon's hormone broth containing 2 per cent peptone, in shallow layers in pint bottles, 20 cc. to a bottle giving a depth of 3/8 inch. The bottles were shaken once a day. The cultures were finally filtered through Berkefeld (N) candles. The pH and M.L.D. of the toxin for rabbits were determined at regular intervals.

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* Edythe Claypole Memorial Fellowship.

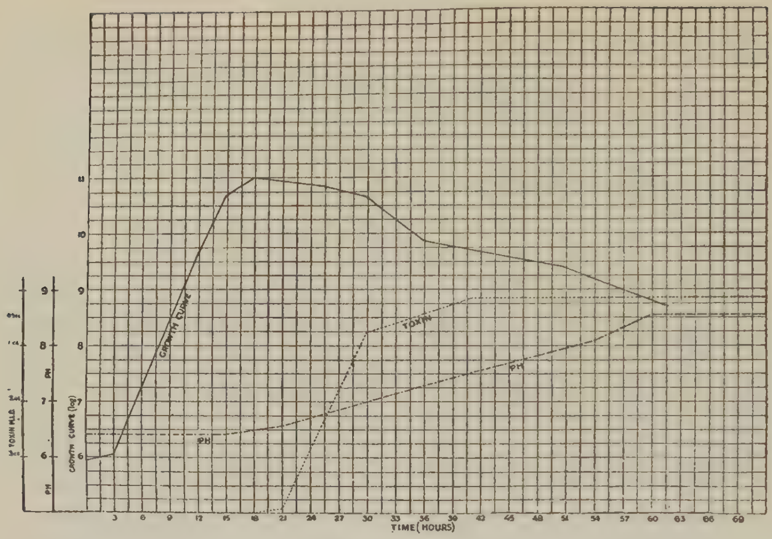

CHART 1.
Relationship between toxin production, growth curve, and pH.

As shown in Chart 1, the toxicity of the broth filtrate begins to rise after the growth curve has exhibited a tendency to descend, and after the pH has risen above 7.0. In several experiments, the peak of the toxin curve was reached when the pH was between 8.0 and 8.6, then the toxicity remained constant for several days.

Rabbits inoculated intravenously appeared sick within 2 to 6 hours. The animals were weak and depressed; the breathing was labored and rapid, and there was profuse watering of the eyes. The dyspnea increased until the respiration showed marked wheezing and evidence of much moisture in the lungs and trachea. As a rule the animals died in from 5 to 24 hours.

On postmortem examination the lungs were large, moist, dark red and firm. The cut surface was very wet, and edematous fluid could be expressed from the alveoli and bronchi. Histologically, the alveoli and bronchi were filled by a finely granular material which in some places compressed the bronchi to a narrow slit. There was marked engorgement of the blood vessels, with small hemorrhages into the interalveolar regions. The trachea was reddish black in color, the vessels being markedly injected; the thymus showed extensive extravascular hemorrhages. The heart was dilated, the right ventricle more than the left. The other organs presented the appearance of cloudy swelling and congestion.

The blood of the rabbits, receiving gradually increasing doses of the toxin, transferred no passive immunity, but the animals acquired a marked tolerance, and withstood in some of the tests as much as 10 M.L.D of the toxin.

The toxin frequently resisted boiling for 5 minutes.

Toxic Substance B. The organisms were grown semi-anaerobically in test tubes for 24 hours, then centrifugalized for one hour at high speed and the supernatant fluid injected intravenously. Three to five cc. of the supernatant fluid killed rabbits in from 6 to 24 hours. Passage through a Berkefeld filter removed the toxic properties. Intravenous inoculation into rabbits was followed by an incubation period of from 1 to 2 hours. The animals exhibited weakness, muscular relaxation, diarrhea, slight watering of the eyes, and died suddenly in convulsions, usually within 7 hours. This toxic substance had an aggressine-like action. It was not antigenic. Rabbits, inoculated with gradually increasing doses, lost weight; they failed to develop any degree of resistance. Autopsy findings, for the most part, showed merely engorgement of the intestinal vessels. This substance is probably identical with the toxic products in young cultures, reported by Zinsser and collaborators[1] for the streptococcus, dysentery and typhoid bacilli, and other organisms.

Summary. Cultures of a strain of Friedlander's bacillus were found to yield two toxic substances; one, produced in aerobic cultures, which has a selective affinity for the lungs causing small pulmonary hemorrhages and an intense edema; the other, produced under semi-anaerobic conditions of growth, is probably a non-specific toxic product possessing an aggressine-like action.

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[1] Zinsser, H., Parker, J. T., and Kuttner, A., PROC. SOC. EXP. BIOL. AND MED., 1920, xviii, 49.

3460

Pharmacology of Veratrum Californicum.

P. J. HANZLIK AND F. DE EDS.

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco.

Veratrum Californicum Durand has not been previously studied pharmacologically. Botanically, it is closely related to *Veratrum viride* and *Veratrum album,* although certain differences for identification purposes have been described by Viehover, Keenan and Clevenger, and Viehover and Clevenger.[1] The active constituents appear to be alkaloids similar to those of the other veratrums, but these have not been isolated as yet. A study of the drug appeared desirable because of suspected activity from reported poisonings, of its easy availability and abundant supply, which is not true of the other veratrums, and of the increasing interest in the therapeutic possibilities of veratrum for the circulation. Our supply was obtained at Cisco, California, 6000 feet elevation in the Sierra Nevada Mountains. Only the underground portion of the plant was found active. This was used as a 10 per cent tincture prepared from a No. 60 powder according to the Type Process P of the U. S. P. X, using 95 per cent alcohol as menstruum, and compared with a tincture similarly prepared from *Veratrum album* (Caesar and Loretz, Germany).

Results with dogs, rabbits, pigeons and frogs indicate that the actions of *Veratrum Californicum* are qualitatively identical with those of *V. album*, but quantitatively the drug appears less toxic, and intermediate between *V. viride* and *V. album*, the lower toxicity being in its favor for therapeutic purposes. The heart is slowed and the blood pressure lowered by small or therapeutic doses through central vagus stimulation, since the slowing does not occur after section of the vagi or atropine. After the second or third dose, the heart is greatly accelerated and the blood pressure raised, continued doses eventually causing collapse and death from direct cardiac depression and paralysis. The respiration is typically slowed or inhibited with toxic doses. Provisionally, the M.F.D in frogs appears to be about 1.75 mgm. per gm. body weight, the definitely effective dose by vein in pigeons and mammals being about 5 mgm. per kilo (0.05 cc. of the 10 per cent tincture), which may slow the pulse 50 per cent and lower the blood pressure (mammals) 40 per cent, and the fatal dose, 37 to 60 mgm. per kilo (0.37 to 0.6 cc. of a 10 per cent tincture). Further work is in progress. This is a preliminary report.

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[1] Viehover, A., Keenan, G. L., and Clevenger, J. F., *J. Am. Pharm. Assn.*, 1921, x, 581; Viehover, A., and Clevenger, J. F., *ibid.*, 1922, xi, 166.

3461

Dissociation Constants of Hexosephosphoric Acids.

LAWRENCE IRVING* AND ERNST FISCHER.

From the Institut für vegetative Physiologie der Universität, Frankfurt a. M., Germany.

Hexosediphosphoric acid from yeast and hexosemonophosphoric acid from muscle (lactacidogen) were kindly made available from preparations by Professor Embden. Electrometric titration showed both stronger than pure phosphoric acid in the region of the first and, more conspicuously, in that of the second dissociation constant. In each case hexosemonophosphoric acid was the stronger.

From titration data with pure phosphoric acid a second constant at 18 degrees of pK_2 equals 6.82 was calculated, agreeing with the usual values given.

For hexosemonophosphoric acid the value similarly determined gave $pK_2 = 6.12$.

For hexosediphosphoric acid only an apparent value of the constant corresponding to the actual third and fourth together was determined at $pK_2 = 6.25$, corresponding with the value of Meyenhof and Suranyi of pK_2, 6.2.

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* National Research Fellow in the Biological Sciences (1925-26).

3462

Influence of Thyroid and Insulin Treatment on Lactacidogen Synthesis in Muscle.

LAURENCE IRVING* AND H. J. DEUTICKE.

From the Institut für vegetative Physiologie der Universität, Frankfurt a. M., Germany.

Rabbits fed with 0.9 grams of dried thyroid tablets daily, with an abundant diet, showed rapid emaciation, followed by death in an exhausted state after 1 to 4 weeks. The muscle brei from such animals shows a great reduction, or almost complete failure of the ability to cause the disappearance of inorganic phosphoric acid under the influence of sodium fluoride. This phosphoric acid disappearance has been shown by Embden and his coworkers to result from its synthesis with carbohydrate into a hexosephosphoric acid ester, termed by Embden lactacidogen. It has been shown in the same Institute that impairment of the synthesis accompanies exhaustion, and certain pathological conditions involving muscular weakness. In the experiments here reported, the greatly reduced synthesizing ability appears associated with the diminution of muscle lactic acid, lactacidogen, and glycogen to almost negligible amounts.

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* National Research Fellow in the Biological Sciences (1925-6).

Muscle brei of animals dying after insulin convulsions also suffered a great loss in synthesizing ability. The same result followed insulin treatment of animals already subjected to thyroid feeding. Under all these conditions the carbohydrate reserve is reduced to minimum values.

Hoet and Marks[1] observed the connection between rapidity of appearance of postmortal rigor and depleted glycogen, lactacidogen and lactic acid content of the muscles of such animals. Normally the postmortal decline in synthesizing ability is relatively slow. In our experiments the residual synthesizing ability immediately after death diminished to hardly detectable values within a half hour or an hour after the rapid onset of rigor.

Soluble starch was used for the necessary source of carbohydrate to effect the synthesis. Experiments with normal animals and the fact of the observed small synthesis usually requiring much more carbohydrate than the minimal muscle glycogen could supply indicate that the wheat starch is utilizable.

In these experiments it is impossible to distinguish whether carbohydrate lack or impairment of synthesizing ability may be regarded as cause of the condition. It is likely that each would contribute to the other, and so initiate the decline in vitality which finally leads to complete exhaustion of the animals from failure of the proper carbohydrate necessary for maintenance of a living muscle system.

This is a preliminary report.

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[1] Hoet, J. P., and Marks, H. P., *Proc. Roy. Soc.* (London) B, 1926, C, 72-86.

3463

Induction of Precocious Sexual Maturity in the Mouse by Daily Pituitary Homeo and Heterotransplants.*

PHILIP E. SMITH AND EARL THERON ENGLE.
From the Department of Anatomy, Stanford University.

Homeotransplants. Sexual maturity is induced in the female mouse by daily pituitary homeotransplants even more rapidly than in the rat. In the rat 8 to 10 daily transplantations are required to bring about sexual maturity at the weaning date (22nd day of life); if the transplants are commenced at about the weaning date, 4 to 6 transplantations only are necessary.[1] In the mouse 3 transplantations made on successive days induced sexual maturity whether begun at the weaning date (20th day of life) or 3 days previously. The structural and physiological changes resulting from this treatment are those characteristic of normal sexual maturity. The vaginal canal is established and contains the cells typical of oestrus, the uterus is engorged, and many large follicles and corpora are present.

In the immature male mouse, as with the male rat, the response of the genital system to the pituitary transplants is much slower than with the female. Four daily transplantations into males varying from 18 to 22 days of age give no, or only a slight increase in size of the testes, and but a small increase (about 25 per cent) in the remainder of the genital system. When the transplants are given even for 17 successive days the testes do not show the marked weight increase over the untreated control that is shown by the remainder of the genital tract, the latter being some 10 times heavier than that of the controls. Even though the testes show, as compared with the rest of the genital system, only a relatively small increase in weight from the transplantations, nevertheless it appears that the implanted pituitary affects the genital tract through the intermediary of the testes, for in their absence no growth response results in the remainder of the genital system.

Heterotransplants. Pituitary transplants from the rat, guinea pig and rabbit also hasten sexual maturity in the mouse. If begun in female mice at the age of 18 days, 3 daily transplants from the adult rat or guinea pig will bring about sexual maturity with the

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* Aided by a grant from the National Research Council Committee for Research in Problems in Sex.

same structural changes as in the homeotransplants. Transplantations of the rabbit pituitary accelerates sexual maturity even more rapidly, the genital system being mature after two transplantations begun in animals 17 days old. In two such animals only 36 hours elapsed between the first transplantation and the opening of the vagina. When only one half the rabbit pituitary was transplanted, however, the usual three daily transplantations were necessary to induce sexual maturity, suggesting that the rapidity of maturing is correlated with the amount of pituitary tissue implanted.

The anterior, not the posterior, lobe of the pituitary, when transplanted, hastens sexual maturity.

Mating. Physiologically as well as structurally, the changes characteristic of sexual maturity are brought about in the mouse by the pituitary transplants many days before they would normally appear. This is shown by the fact that these precociously developed animals will mate. In two mice receiving rabbit pituitary transplants, the only two thus far tested, mating, as evidenced by the presence of a plug and sperm in the vagina, took place on the 19th day of life, and within 48 hours after the first pituitary transplantation had been made.

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[1] Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 131-132; *Am. J. Physiol.*, 1927, lxxix, 114-125.

3464

The Regeneration of Rodent Peridental Membrane.*

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From the Department of Bacteriology and College of Dentistry, University of California.

The work[1, 2] in which one of us (W. C. F.) has participated indicates that a certain degree of repair of peridental fibrous tissue in man may follow careful treatment of the pyorrheal pocket. Many points regarding the sequence of events in regeneration of peridental membrane, however, were left without explanation. Thus, it was decided to examine the possibilities of repair of this peridental structure in the experimental animal.

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* This work was done in connection with the original California Stomatological Research Group. It was supported in part by funds from the Carnegie Corporation, the American Dental Association and the Associated Radiograph Laboratories of San Francisco.

If a dental bur be passed through the gum and alveolar process into the incisor tooth there is produced a local injury which includes destruction of the external soft tissue, alveolus, peridental membrane, cementum and dentine in order of sequence. If a series of animals be subjected to this operation, they may be sacrificed at varying periods afterwards, in order that an appropriate spacing may be obtained for subsequent examination regarding the various steps of destruction and repair which follow in each of the structures included. For this purpose we selected the rabbit and the guinea pig for a particular reason. The rodents are provided with continuously growing teeth. As a result the various structures will change in relationship for as the tooth continues to grow, an uninjured tooth surface will come opposite to the site of injury to the alveolar bone. For histological examination this presents advantages, since peridental repair is more likely to take place from an intact dental surface than from one which is fractured.

Two series of rabbits and two series of guinea pigs have been submitted to operation as described in the preceding paragraph. Animals thus treated have been sacrificed at semi-weekly intervals over a period of 6 weeks. The tooth with its adjacent structures was sawed out immediately after death, fixed, and then prepared in serial longitudinal sections in celloidin by methods which are described by one of us (A. W.).[3] We have used both haematoxylin-eosin and Van Geisen stains for these.

Our results from these series of observations may be summarized as follows. Infection follows this operation in nearly all instances. The infectious process, together with the presence of necrotic detritus, stimulates the appearance of multinucleate osteoclasts within three days time. These break down alveolar bone, and this destruction is initiated on the inner surface of the process at a point just apical to the site of injury. Osteoclastic activity then spreads to other portions of the osseous structure but there is a decidedly obvious tendency for it to extend towards the apex. The destructive process, therefore, appears to follow the lymphatic stream rather than the main channels of circulation. Lysis of alveolar bone is attended by severing of peridental connective tissue. Subsequent to bone destruction, the peridental fibres which formerly were a portion of the bone at this point are also destroyed.

Attempts at regeneration of the peridental connective tissue fibres are evident during a period varying between 3 and 7 days after their destruction in each of the species of our experimental animals. The development of peridental fibres marks its inception by the appearance of fibroblasts upon the cemental surface. These then undergo the usual fibroblastic development attended by lengthening of the nucleus and cytoplasm in a manner similar to that found in other portions of the body. The point of especial interest lies in the fact that growth of this tissue is begun at the tooth surface rather than from the alveolar surface. This is contrary to certain previous conceptions.

After reconstruction of peridental fibres has begun at the cemental surface, repair of bone commences. Osteoblasts appear. Cancellous bone is laid down about a fibrous net work. Fusion of fibres already formed precedes bone formation. Colloidal precipitation attends it as proven by the stain reaction of Van Giesen technique. Thus, following the genesis of the fibres at the cementum, the gap across to the bone is spanned and now the fibre becomes incorporated in the reforming alveolar process. Repair thus may proceed to completion.

This is a preliminary report.

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[1] Hanford, W. H., Patten, C. O., Westbay, C., and Simonton, F. V., *Dental Cosmos*, 1923, lxv, 12-17.

[2] Fleming, W. C., A Clinical and Microscopical Study of Peridental Tissue Treated by Instrumentation. Paper delivered at Pacific Dental Conference, Portland, Oregon, June 25, 1926.

[3] Williams, Adrienne, *Dental Cosmos*, in press.

3465

Pulse Pressure, Its Probable Relationship to Stroke Volume.

J. MARION READ.

From the Division of Medicine, Stanford University Medical School.

Numerous investigators have shown that in hyperthyroidism the tachycardia varies in degree directly with the metabolic rate. It seems reasonable to suppose that this increased heart rate is an adaptation for increasing the cardiac minute volume, since the minute volume is known to be increased in this condition. Previously[1] I have pointed out that the pulse pressure also varies directly with metabolism. In Table I are shown the values obtained in 1000 observations of the pulse rate, blood pressure and metabolic rate made simultaneously under basal conditions. It will be observed that the rise in pulse pressure with increasing b.m.r. is due to rise in systolic pressure, since the diastolic pressure remains practically constant.

The pulse rate and pulse pressure both increase in a parallel manner with elevation of the basal metabolic rate.

When gathering these data it was observed that some subjects showed a disproportionate increase in either pulse rate or pulse pressure, with little or no change in the other. This suggested that some subjects might increase the minute volume by increasing the rate of output per minute, while others accomplished the same increase in total blood flow by increasing the volume ejected into the aorta at each systole. Pulse pressure might then be a rough measure of stroke volume.

Recently there came under observation two individuals with complete heart block, as shown by electrocardiogram, neither of whom suffered limitation of activity imposed by circulatory insufficiency. One was a woman, 47 years old whose slow pulse had excited interest since girlhood. P. R. 36-44, blood pressure, systolic 180, diastolic 80. The other was a man, 70 years old. P. R. 28-30. Systolic pressure, 200-240; diastolic, 70-100. Neither had aortic insufficiency.

The normal diastolic pressures in these subjects and the lack of any circulatory embarrassment, with moderate cardiac enlargement suggested that the blood pressure changes were adapted to permit an adequate minute volume even with a pulse rate reduced to about half the normal. The wide pulse pressure in these cases I interpret as

TABLE I.

The mean values for blood pressure and pulse rate as they vary through the normal and pathologic ranges of basal metabolic rate.

Basal metabolic rate	Number of observations	Blood Pressure		Pulse pressure	Pulse rate
		Sytolic	Diastolic		
120.1 to 130	1	166	89	77	159
110.1 to 120	1	170	60	110	132
100.1 to 110	1	154	70	84	121
90.1 to 100	2	152	77.5	74.5	116
80.1 to 90	3	136.7	78	58.7	122
70.1 to 80	12	148.6	70	78.4	111
60.1 to 70	32	138.2	72	66.1	109.1
50.1 to 60	48	133.7	71.2	62.3	104.4
40.1 to 50	76	130.8	73.2	58.5	100.8
30.1 to 40	110	126.4	73.2	53.1	93.3
20.1 to 30	123	122.9	71.0	51.1	83.3
10.1 to 20	142	118.0	73.0	45.1	79.8
0.1 to 10	152	110.3	68.5	42.4	72.5
-9.9 to 0	138	107.7	69.2	38.8	68.9
-19.9 to -10	117	108.2	71.3	36.8	65.7
-29.9 to -20	37	106.9	73.3	32.9	62.7
-39.9 to -30	5	97.2	66.4	30.8	58.4
Total	1000				

indicating increased stroke volume and believe that, with a normal diastolic pressure, pulse pressure variations indicate variations in stroke volume and that a reciprocal relationship exists between pulse pressure and pulse rate if the minute volume remains constant. This is a preliminary report.

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[1] Read, J. Marion, *Arch. Int. Med.*, 1924, xxxiv, 553.

3466

The Use of the Cathode Ray Oscillograph for Electrocardiography.

WILLIAM DOCK. (Introduced by Ernest C. Dickson.)

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In order to time the point in the cardiac cycle at which a number of rapid roentgen ray films of the heart are taken, it was wished to develop a small portable electrocardiograph with no string or delicate part to be damaged by the currents from the X-ray circuit. The cathode ray oscillograph, as first used by Erlanger and Gasser,[1] has proved quite satisfactory for making electrocardiographs. Gasser[2] found that with his amplifier the heart currents gave lower curves than similar voltages in nerve preparations, due to the low resistance of the body as compared with the resistance of the amplifying tube. The apparatus with which this record was made differs from that of Gasser and Erlanger (a) in that the same source of current is used for cathode ray tube and amplifiers; (b) the sensitive film moves over the face of the tube giving a continuous curve instead of standing curves; (c) four amplifying tubes are used, the first three with amplification constant 20, the last a UX112 tube with a constant of 7; (d) one lead goes directly to the filament of the first tube, the other passes through a 2 microfarad condenser to the grid, which connects to the filament through a one megaohm leak. The apparatus gives curves with a motion of one cm. per one millivolt input and at rates of motion of film up to fifteen cm. per second.

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[1] Erlanger, J., and Gasser, H. S., *Am. J. Physiol.*, 1924, lxii, 496-524.

[2] Gasser, H. S., personal communication.

Illinois Branch.

(With the Biological Club.)

University of Chicago, March 1, 1927.

3467

Transformation of Lymphocytes of Thoracic Duct into Polyblasts (Macrophages) in Tissue Culture.*

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From the Department of Anatomy, University of Chicago.

The ability of the lymphocytes of the blood stream to develop into either myeloid or phagocytic cells is denied by most investigators. In a long series of experiments Maximow[1] showed that the lymphocytes and monocytes of the blood wander into the tissues in inflammation and transform there in a very short time into exudate mononuclear cells or polyblasts, as he called them. A portion of the latter he also derived from his resting wandering cells or histiocytes. These, as he showed, have a close embryogenetic relationship to the lymphocytes.

Several investigators have studied the development of lymphocytes and lymphoid tissue in tissue culture. Their results have not been uniform or decisive because it was very difficult to follow the fate of the various kinds of cells present in the explanted tissues.

At the suggestion, and with the aid of Dr. A. Maximow, I cultivated the lymph of the thoracic duct of the rabbit. This fluid in the rabbit contains only lymphocytes, or at most an occasional monocyte. (Simpson,[2] Thorne and Evans,[3] Kindwall.[4]) Lymph was pipetted aseptically from the cervical portion of the thoracic duct of adult rabbits (anesthetized with ether) which had been starved for 24 hours. The lymph was allowed to clot; it was then minced in Ringers solution and explanted in heparin plasma with various tissue extracts and tissues. This report gives the results obtained in cultivating lymph with embryonic extract.

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Except for a few scattered monocytoid cells and erythrocytes, the lymph used in these experiments, as seen in spravital, dry and wet smears, contained only lymphocytes.

After the cultures were studied in the living condition, both with and without supravital staining with neutral red, they were fixed in Zenker-formol, imbedded in celloidin, cut in serial sections, and stained with hematoxylin-eosin-azure II.

Immediately after explantation the cultures contain a compact fibrin clot filled with large numbers of small, medium sized and occasional large lymphocytes with here and there an erythrocyte. After the cultures have become warm in the incubator, the lymphocytes begin to migrate from the clot. After 7 or 8 hours a distinct change is to be seen in the cells within the clot. Many of them have become distinctly larger and contain many vacuoles which stain a brilliant red with neutral red. These vacuoles are usually arranged in typiical rosettes about the indentation of the nucleus. There are numerous transition forms between these cells and the lymphocytes. After 20 hours the lymph clots contain very great numbers of the large cells. These are usually 3 or 4 times the diameter of the original small lymphocytes. The large cells have, as far as one can see in the clot, movable, dull, transparent, membrane-like pseudopodia, and, a large amount of cytoplasm which contains a variable number of bright lipoid drops. As the cultures grow older, the number of these cells increases. Mitotic figures are exceedingly rare. When lithium carmine is added to the cultures the large cells store the dye in large amounts in granular form. After three days there appear a few long, spindle shaped cells.

Shortly after explanation many of the small, medium sized and large lymphocytes become round and degenerate within the clot. Most of the cells which have migrated from the explanted lymph clot also die in the course of the first two days.

The fixed and stained sections of the cultures give a much clearer idea of what has taken place within the explants than has been obtained by the study of the living cultures.

Several hours after explantation the great majority of the lymphocytes begin to show marked changes. They are now much larger than in those cultures fixed immediately after explantation. The relative percentage of medium sized lymphocytes has increased greatly. Mitotic figures are entirely absent at this stage. Concomitantly with the marked increase in size of the cells there appears a distinct loss of basophilia of the cytoplasm. The nucleus of many of the cells becomes much larger and seems more vesicular, in that the coarse chromatin in particles have divided into many smaller ones. The nucleus becomes decidedly bean-shaped because of a one-sided indentation. The nucleolus of the lymphocytes undergoes no change. The small and medium sized lymphocytes of the thoracic duct usually do not contain a demonstrable attraction sphere; occasionally one can see a small acidophilic area opposite the indentation of the nucleus. In the above described hypertrophy of the lymphocytes in the cultures the sphere becomes easily visible, it increases in size and may in a short time become as large as the nucleus. The latter then is excentrically located and seems relatively much smaller than before.

In some of the lymphocytes the described nuclear changes may be somewhat delayed. In these the cytoplasm increases greatly in size while the nucleus remains small and retains its original trachychromatism. In such cells the nucleus-plasma relation shifts markedly in favor of the cytoplasm. The latter may lose its basophilia almost entirely; only the outer edge of the cell body may stain blue. Inside this blue area vacuoles may appear and thin membrane-like pseudopodia may extend from the edge of the cell. The differences between these hypertrophied lymphocytes and the normal cells of the same lymph before and shortly after explantation are particularly striking when studied with the aid of a comparison ocular.

Twenty hours after explantation, the explanted bits of lymph clots contain the debris of many dead lymphocytes and fairly large numbers of actively ameboid, small and medium sized lymphocytes. But the dominant feature of the cultures is the presence of great numbers of large ameboid cells which cannot be differentiated from the exudate mononuclear cells (polyblasts) which are typical for the field of inflammation.

This hypertrophy of the lymphocytes has not taken place in all of our series of cultures of lymph. Our best results have been obtained from those rabbits in which the lymph was water clear and seemed free from fat.

The phagocytopoietic ability of the small and medium sized lymphocytes of the thoracic duct and, implicitly, of the blood stream of the rabbit is clearly demonstrated in these experiments.

This is a preliminary report.

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[1] Maximow, A., *Beitr. z. path. Anat. allg. Pathol.*, 1902, Suppl. V; 1903, xxxiv, 153; 1904, xxxv, 43; 1905, xxxviii, 301; 1906, xxxix, 333; 1907, xli, 122. *Arch. f. mikrosk. Anat.*, 1909, lxxiii, 444.

[2] Simpson, M., *J. Med. Res.*, 1922, xliii, 77.

[3] Thorne, G., and Evans, H., *Anat. Rec.*, 1922, xxiii, 42.

[4] Kindwall, J., *Johns Hopkins Hosp. Bull.*, 1927, xi, 39.

3468

Development of Non-granular Leucocytes (Lymphocytes and Monocytes) into Polyblasts (Macrophages) and Fibroblasts in Vitro.*

ALEXANDER A. MAXIMOW.

From the Department of Anatomy, University of Chicago.

The question whether the non-granular leucocytes of the blood of mammals are mature, specifically differentiated cells, or cells endowed with possibilities of progressive development is still subject to discussion. The prospective potencies of these elements are especially manifest in inflammation.

Maximow[1] has claimed, since 1902, that the polyblasts or mononuclear exudate cells of inflamed tissue arise in part from local fixed cells, the resting wandering cells or the histiocytes of the tissue, but for the major part represent lymphocytes and monocytes, which have migrated from the blood vessels and have undergone rapid hypertrophy in the tissue. This double origin of the polyblasts is quite natural, for a study of the embryonic histogenesis of the connective tissue and blood reveals a close relationship between the histiocytes and the non-granular leucocytes.[2] In later stages of inflammation Maximow found the further transformation of polyblasts into fibroblasts. Thus, as the polyblasts arise in part from lymphocytes, he advocated the possibility of a transformation of lymphocytes and monocytes into fibroblasts.

This idea of a progressive transformation of non-granular blood leucocytes into polyblasts and finally into fibroblasts has met strong resistance. According to the dominant opinion, the polyblasts develop from the cells of the walls of the blood vessels (endothelium, adventitial cells). The monocytes and lymphocytes are usually considered as specifically differentiated elements which are unable to produce any other cell types.

The method of tissue culture has had a decisive influence on the problem under consideration. Several experimenters have shown that the non-granular blood leucocytes can transform themselves into macrophages (polyblasts) and even into "fibroblast-like" cells *in vitro* (Awrorow and Timofejewsky,[3] Carrel and Ebeling,[4] Maximow,[5] Lewis,[6] Fischer[7]). However, the problem is not completely solved as yet. Most of the investigators using the tissue culture

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

method, with the exception of Maximow[5] and Timofejewsky and Benewolenskaja,[8] believe that the lymphocytes are incapable of progressive development, and that the monocytes are genetically independent from the lymphocytes. They accordingly discriminate sharply between these two cell types. The developmental possibilities of the monocytes are admitted, whereas the fate of the lymphocytes *in vitro* remains doubtful (Lewis[6]). Moreover the development of regular connective tissue from leucocytes also has not as yet been observed, only "fibroblast-like" cells were found.

The use of heparin enables us easily to apply tissue culture experiments to animals, in which the plasma clots very rapidly, such as guinea pigs, rats, etc. Guinea pigs seem to be especially favorable for culturing leucocytes. For the direct demonstration of the cellular transformations, in addition to sections of fixed material, the supravital neutral red staining was used.

The progressive changes of the lymphocytes in cultures of the buffy coat of guinea pig blood begin almost at once after explantation. Many of them remain unchanged and later gradually degenerate. But the major part transforms in the course of 6 to 8 hours into typical polyblasts. The nucleus becomes clearer and kidney shaped, the protoplasm increases in quantity and accumulates on one side of the nucleus. Whereas in the blood the lymphocytes show very few small neutral red vacuoles around the cytocentrum, *in vitro* they rapidly develop a large rosette of small and large neutral red vacuoles. The cells display intense ameboid movements with formation of hyaline membrane-like pseudopodia. During the second day the cells increase further in size and accumulate fat droplets. In the meantime the monocytes present in the culture also hypertrophy and accumulate an increasing quantity of neutral red vacuoles and fat. After 2 to 3 days monocytes and lymphocytes indiscriminately have transformed themselves into typical large phagocytic polyblasts. Thus, direct observation proves beyond doubt the rapid development of lymphocytes into polyblasts (macrophages).

A large part of the macrophages of lymphocytic and monocytic origin, during the third day, especially at the periphery of the explant, begin to stretch out and to develop long, spear shaped, nonmotile processes, while the large nucleus acquires a more regular oval shape. At first, however, the condition of these "fibroblast-like" cells must be in a peculiar unstable equilibrium. As soon as they are focused under an immersion lens with a sufficiently strong light, they at once rapidly contract, round off, and resume their former ameboid wandering with highly motile membrane-like pseudopodia. However, after a sufficient length of time (6 days or more) the changes seem to become irreversible. Typical fibroblasts appear and in numerous cultures, especially those growing in bottles, instead of slides, large colonies of typical fibroblasts develop, which cannot be distinguished from cultures of fibroblasts, obtained from regular connective tissue.

Thus, the possibility of the transformation of a small lymphocyte into a macrophage (polyblast) and further into a fibroblast seems to be demonstrated.

This is a preliminary report.

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[1] Maximow, A., *Beitr. z. path. An. u. z. allg. Path.*, 1902, Suppl. v, 1.

[2] Maximow, A., *Arch. f. mikr. Anat.*, 1909, lxxiii, 444.

[3] Awrorow, P., and Timofejewsky, A., *Virch. Arch.*, 1914, ccxvi, 184.

[4] Carrel, A., and Ebeling, H., *J. Exp. Med.*, 1922, xxxvi, 365.

[5] Maximow, A., *Klin. Wochenschr.*, 1925, iv, 1486.

[6] Lewis, M., *Am. J. Path.*, 1925, i, 91.

[7] Fischer, A., *Compt. rend. soc. biol.*, 1925, xciii, 109.

[8] Timofejewsky, A., and Benewolenskaja, S., *Arch. f. exp. Zellforsch., bes. Gewebezüchtung* (Explantation), 1925, ii, 31.

3469

Experimental Obstruction of the Mesonephric Ducts.

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From the Department of Anatomy, University of Illinois College of Medicine.

In a previous paper[1] the writer showed that destruction of the growing ends of the Wolffian ducts in young chick embryos resulted in the failure of these ducts to establish connection with the cloaca. Under these circumstances, the Wolffian bodies became hydronephrotic, the cloaca failed to differentiate a urodaeal sinus, the allantois remained rudimentary, and the chicks died not later than the end of the sixth day from either lack of oxygen or a possible retention of urine. Since up to that time the allantoic fluid of normal embryos had never been analyzed by modern microchemical methods, the latter possibility could not at first be determined. But investigations begun in 1924[1] have shown that the end product of nitrogen metabolism in chick embryos is uric acid; that measurable amounts of it appear in the allantoic fluid as early as the fifth day of incubation; that it is eliminated in rapidly increasing amounts up to at least the 15th day[2]; and that during all this time, from the 5th to the 15th day) the mesonephric tubules undergo rapid increase in both length and volume.[3]

The present article deals with the effect of experimental obstruction of the Wolffian ducts upon the nephrogenic tissue of the embryo. In this connection it should be recalled that the rudimentary pronephros gives rise to the Wolffian ducts; that from the 20th to the 30th somites these ducts are joined by the definitive mesonephric tubules; and that after the lower end of each duct has given rise to the metanephric diverticulum the latter is joined by metanephric tubules derived from the nephrogenic tissue of somites 31 to 33 (Lillie).

In all cases in which the writer has prevented one or both Wolffian ducts from reaching the cloaca, the ureter on that side has never formed, and no metanephric tubules have developed. There is, however, a concentration of nephrogenic tissue medial to the posterior cardinal vein (*Bl.,* Fig. 3). But this blastema never differentiates nor becomes as dense as nephrogenic tissue, which is in contact with the pelvis of the ureter (cf.*Bl,* and *Met,* Fig. 2, from a unilateral case of hydronephrosis).

Similarly, the mesonephric portion of the nephrogenic tissue never differentiates into tubules in the absence of the Wolffian duct from a given region. This is especially easy to demonstrate when the original lesion which destroys the growing ends of the Wolffian ducts is below the level of the vitelline veins (22nd somite). Above this level the operation is much more difficult, but I have three such cases. The first is shown in Fig. 3, from a chick operated upon at 39 hours and then reincubated four days longer, the downgrowth of the Wolffian ducts having been stopped at the level of the wing-bud (17 to 19 somites) just above the somites which give rise to the definitive Wolffian body. In this photomicrograph, which is taken at the level of the caeca, neither right nor left Wolffian *(W.b.)* contains any tubules below the lesion. In all other respects the embryo appears to be normal. A second case is shown in Fig. 1, a chick of 66 hours incubation, fixed 28 hours after the operation. This shows a section taken at the level of the 23rd somite, 4 somits below the lesion (*cf.* Lillie, 1908, Fig. 111). In this earlier stage a slight concentration of the nephrogenic tissue (Nph.) can be seen medial to the posterior cardinal veins *(P.v.)* but this apparently disappears long before the sixth day (Fig. 3). From these observations it thus appears that neither the mesonephric nor the metanephric tubules develop in the absence of the Wolffian duct from territory adjacent to the appropriate nephrogenic tissue. The rudimentary pronephros must therefore be considered a vital organ, for without the Wolffian ducts to which it normally gives rise, neither the embryonic nor the adult kidney is formed.

In conclusion, I wish to record the probable occurrence of compensatory hypertrophy of the excretory gland of the embryo, following unilateral hydronephrosis. Such a case is shown in Fig. 2.

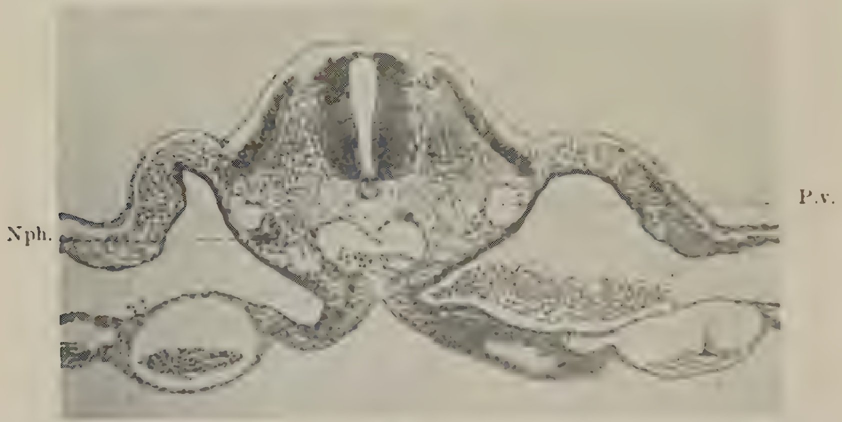

FIG. 1.

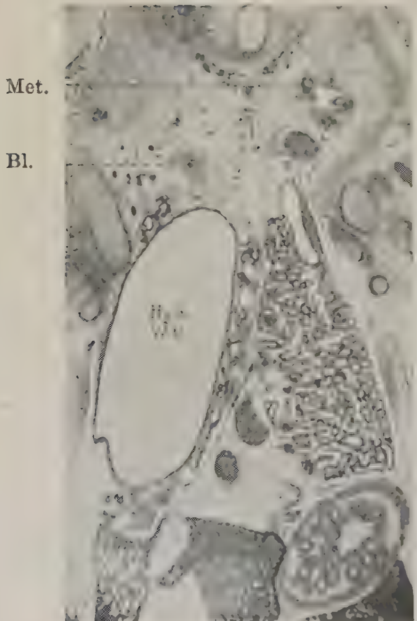

FIG. 2.

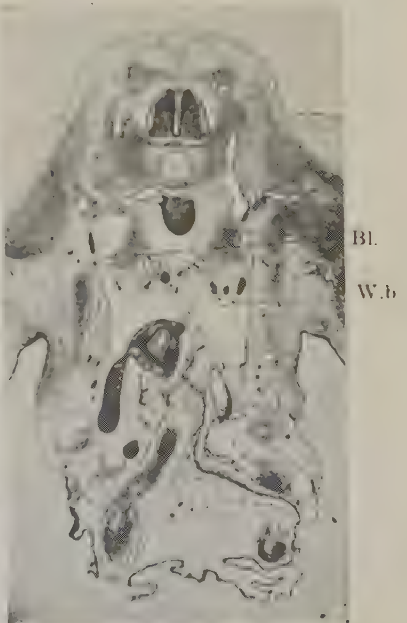

FIG. 3.

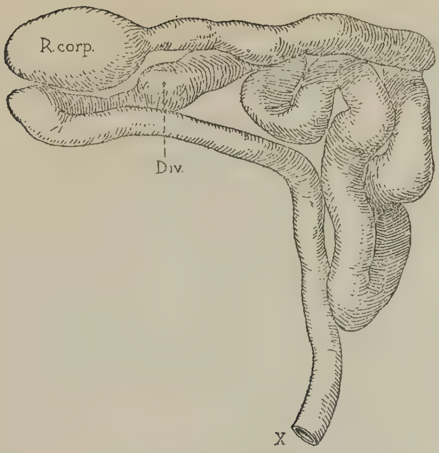

FIG. 4.

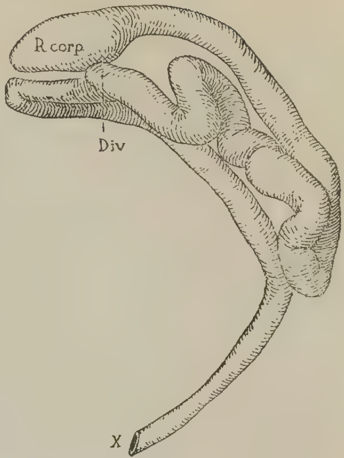

FIG. 5.

Wax model of mesonephric tubule taken from widest portion of left Wolffian body of hydronephrotic embryo shown in Fig. 2, (6 days, 9 hrs. 17.3 mm.) X 85

Mesonephric tubule from widest portion of left Wolffian body of a normal embryo (H. E. C., 2076) incubated 6 days and 7 hrs., 17.3 mm. X 85.

R. Corp., renal corpuscle; *Div.*, diverticulum separating secretory tubule from mesonephric ureter; *X*, junction of ureter with Wolffian duct.

The growing end of the right Wolffian duct of this embryo was destroyed at the level of the 30th somite (50 hours), the duct on the left side being untouched. After 4¼ days of incubation the embryo was fixed. The sections showed an enormous distention of the right mesonephros (*Hyd. W.b.*, Fig. 2). The one on the left side, however, appeared to be normal. But when it was modelled in wax and its volume obtained by displacement, it was found to be just twice as large as the left mesonephros of a normal embryo of the same age and length in the Harvard collection. When the tubules from these embryos were modelled, the corpuscle and secreting portion of the operated embryo were found to be 1.86 times the volume of the corresponding parts of a normal embryo (Figs. 4 and 5). Since, however, the operated embryo seemed older than the other, despite its similar length and age, the relative size of the two embryos was tested by modelling the livers of the two specimens. The volumes of these organs were found to be in the ratio of 1.6 to 1, proving that the operated embryo had grown faster than the other, but also showing that the left Wolffian body of the operated embryo had grown faster than the liver. As an additional check the glomeruli (inner capsules) of 6 mesonephric tubules were modelled and compared with 6 glomeruli from a normal 11-day chick which had already been modelled for another purpose. Those from the operated embryo (6 days and 9 hours, 17.3 mm.) were found to be larger than those from the 11-day chick, which measured 31 mm., the average volumes of the glomeruli being in the proportion of 1.2 to 1. It seems reasonable to assume, therefore, that we are dealing with a compensatory hypertrophy of the embryonic kidney which is not unlike that of the adult organ. Also, it is believed that this similarity of reaction to pathological conditions, coupled with the similarity of end products of nitrogen metabolism found in embryo and adult (namely uric acid), demonstrates a greater homogeneity of these serial excretory organs than has been recognized hitherto.

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[1] Boyden, E. A., *J. Exp. Zool.*, 1924, xl, 437-472.

[2] Fiske, C. H., and Boyden, E. A., *J. Biol. Chem.*, 1926, lxx, 535-556.

[3] Boyden, E. A., and Fiske, C. H., *Anat. Rec.*, xxxiv, No. 6, in press.

3470

Relations between pH, Agglutination and P. D. with Bacterium Phaseoli Sojense.

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In the course of a series of studies on a group of bacteria pathogenic for plants, it was found that a strain of *Bacterium phaseoli sojense,* when plated on agar, gave colonies that were distinctly "Rough" and "Smooth". The cultural characteristics, virulence for plants and the serological reactions of these organisms have been described elsewhere.[1] We were interested in studying the agglutination reactions of these cultures, particularly because the "Rough" organisms, although more agglutinable than the "Smooth", as is generally the case, had also been found to be spontaneously agglutinated even in distilled water suspensions and after repeated washings with water.

In Table I we present the results of agglutination and P. D. (electrophoretic potential difference) measurements on suspensions prepared in distilled water after three washings with water.[2] Adjustments of pH were made by appropriate additions of aqueous HCl or NaOH solutions to the specified pH values.

TABLE I.

The influence of pH on the agglutination and P. D. of washed Bacterium phaseoli sojense in distilled water.

Smooth			Rough		
pH	Agg.	P. D.	pH	Agg.	P. D.
1.2	+	— 3.9	1.2	+	+ 3.2
1.4	+	+ 1.6	1.4	+	0
1.6	++	+ 3.9	1.6	+	+ 12.5
1.8	++	+ 7.55	1.8	+	+ 14.4
2.	++	+ 11.1	2.	+	+ 19.4
2.2	++	+ 11.9	2.2	++	+ 21.4
2.4	++	+ 13.7	2.4	+++	+ 13.8
2.6	++	+ 11.4	2.6	+++ C	+ 2.6
2.8	++	+ 10.9	2.8	+++	— 5.3
3.1	+	— 15.1	3.	++	— 18.
3.5	—	— 46.1	3.2	++	— 22.5
4.1	—	— 63.1	3.4	++	— 27.1
4.6	—	— 82.8	3.6	++	— 34.7
5.0	—	—104.1	3.8	++	— 42.8
5.3	—	—113.2	4.	++	— 42.7
5.6	—	—132.6	4.2	++	— 47.6
6.0	—	—146.3	4.4	++	— 48.1
6.4	—	—168.9	4.6	+	— 48.3
6.8	—	—147.1	4.8	+	— 47.4
7.2	—	—113.8	5.	+	— 48.1
7.6	—	—132.0	5.2	+	— 39.4
8.0	—	—149.4	5.4	+	— 38.7
8.4	—	—161.2	5.6	—	— 48.3
8.8	—	—137.2	5.8	—	— 52.3
9.3	—	— 82.1	6.	++	— 19.1
9.6	—	— 99.9	6.2	+++	— 85.0
10.	—	—118.6	6.4	+++ C	— 85.0
			6.6	+++ C	— 82.0
			6.8	+++ C	— 77.0
			7.	+++ C	— 85.0
			7.2	+++ C	— 92.8
			7.5	+++ C	— 86.6
			7.6	+++ C	— 73.0
			7.8	+++ C	—106.9
			8.0	+++ C	— 82.6
			8.2	+++ C	—119.7
			8.4	+++ C	— 88.7
			8.6	+++ C	— 90.7
			8.8	+++ C	— 83.7
			9.0	+++ C	— 85.3
			9.2	+++ C	—112.8
			9.4	+++ C	— 87.6
			9.6	+++ C	—117.4
			9.8	+++	— 98.2
			10.	++	—104.0

C. Cloudy, almost complete agglutination.
+ Slight agglutination.
++ Partial agglutination.
+++ Nearly complete agglutination.
++++ Complete agglutination.

pH, measured colorimetrically. Agglutination, measured macroscopically. P. D., expressed in micra per second for an impressed voltage of 110 volts.

From an inspection of the data in Table I it appears that:

1. The "Rough" strain is, and the "Smooth" strain is not spontaneously agglutinable in distilled water.
2. Both strains showed marked acid agglutination.
3. Only the "Rough" showed alkali agglutination.
4. In the acidulated solution there is correlation between the effects of acidulation upon agglutination and upon reduction of the negative P. D. to approximately zero (isoelectric) or to slightly positive values.
5. There is no definite correlation between P. D. and agglutination in alkaline solutions.
6. The "critical" potentials are different for the "Rough" and "Smooth" strains.

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[1] *Cf.* Sharp, *Bot. Gaz.* (in press). Link and Sharp, *ibid.*

[2] *Cf.* Falk, Gussin and Jacobson, *J. Inf. Dis.*, 1925, xxxvii, 481-494.

3471

Production of Toxic Substances in Vitro by Fusarium Lycopersici.

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From the Hull Botanical Laboratory, University of Chicago.

The purpose of this research was to determine how the pathological effects characteristic of wilt of the tomato *(Lycopersicon esculentum)* are produced. This disease, which is caused by the filamentous fungus, *Fulsarium lycopersici,* is characterized by gradual or sudden loss of turgor in the leaves and stems, and generally by eventual dessication and death. Internally, the most characteristic symptom is a decided vascular discoloration (necrosis). Precedent to, or accompanying loss of turgor, local or general chlorosis of the leaves often occurs. In fact, at times, when environmental factors, particularly temperature, are not optimal for the pathogen chlorosis, and resultant retarded or arrested growth of the plant are the outstanding or only external symptoms, so that the disease resembles a show blight.

The pathogen lives primarily in the xylem elements of the host. Following the early work of Smith,[1] who established that wilting in bacterial wilt of cucurbits is essentially due to a clogging of the vessels, it has been assumed quite generally that in such vascular mycoses as tomato wilt and cabbage yellows, the parasite affects the host by clogging the water conducting elements and thus mechanically interfering with the water supply of the transpiring top of the plant. A considerable body of observational and experimental data, especially by Clayton,[2] suggests that this hypothesis alone is not adequate. A different, or an additional possibility is that the fungus produces or induces the production of substances which exert a slow and progressive, or a sudden and rapid toxic effect on the cells of the host.

In these experiments we were especially concerned in testing the validity of the second hyphothesis. Pure cultures of the fungus were grown for various lengths of time in various volumes of a synthetic medium which consisted of 1,500 cc. of H_2O, 50 grams of $C_{12}H_{22}O_{11}$, 10 grams of KNO, 5 grams of KH_2PO_4, 2.5 grams of $MgSO_4$, and a trace of Fe_2Cl_3. The fungus was filtered off and the filtrate passed through Berkefeld filters. Next, vigorous tomato plants were cut off just above the soil and placed in the culture filtrate and in aqueous dilutions (1-1, to 1-16) of the culture filtrate. Forty plants were thus set up, each in a separate test tube. The controls consisted of tops placed in (1) the nutrient medium, (2) distilled water, and (3) tap water. Fifty plants were used as controls. Each of the control plants in the nutrient medium remained unchanged and some of those in distilled and in tap water even developed adventitious roots. All of the plants placed in the culture filtrate developed typical symptoms of tomato wilt within varying lengths of time depending upon the concentration of the culture filtrate and the age of the culture from which the filtrate was derived. In general, with a given volume of culture medium, the older the culture the more rapid the yellowing and wilting. Furthermore, no wilting was obtained when the culture filtrate was diluted with more than 4 volumes of distilled water.

Next a series of experiments was run to determine whether plants of wilt-resistant varieties would be affected by the toxic culture filtrate. Plants of both wilt susceptible (John Baer, Bonnie Best) and wilt-resistant varieties (Marbanna, Marbellosa, and Norton) were placed in the filtrates. Forty plants of the former varieties were used and 30 of the latter. The resistant varieties wilted as readily as the susceptible ones.

To determine the effect of the toxic element upon plants with unbroken root systems the filtrate was applied to the soil of plants grown in garden soil and in pure quart sand. Walter culture plants also were arranged so that the root systems were immersed in the culture filtrate. In the controls, nutrient medium was substituted for the culture filtrate. After 48 hours the potted plants, to which the culture filtrate had been added, had wilted. The controls were healthy even on the fifth day. The seedlings grown in water cultures wilted in 48 hours after transfer to the culture filtrate while those transferred to the nutrient medium remained healthy.

To determine whether the killing effect of the culture filtrate is specific for the tomato plant, cuttings of the following plants were placed in the culture filtrate: Selaginella sp., cotton, sunflower, garden bean, and mustard. All of these wilted in 48 hours.

The toxic element is not destroyed by prolonged boiling of the culture filtrate. It is not carried over in steam sterilization. A water extract of the residue from complete evaporation of the filtrate on a water bath, is still toxic.

It is apparent from these experiments that *Fusarium lycopersici* produces a thermo-stabile toxic element *in vitro* which, when introduced into tomato plants, produces typical wilt symptoms. Studies of the nature of the toxic element, of the mechanism of its effects on the host, and of other related problems are in progress.

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[1] Smith, E. F., Bacteria in Relation to Plant Diseases, Vol. II, 1911.

[2] Clayton, E. E., *Am. J. Bot.*, 1923, x, 71-88.

3472

Organic Chlorides of Tissues and Possible Relation to Gastric Hydrochloric Acid Formation.

MARTIN E. HANKE AND PAUL B. DONOVAN. (Introduced by F. C. Koch.)

From the Department of Physiol. Chemistry, University of Chicago.

Among the current theories for the chemistry of the gastric hydrochloric acid mechanism, there are none which allow for the production of the acid without a coincident *local* formation of an equivalent amount of alkali. Since there is no evidence that gastric tissue becomes alkaline during acid secretion, and since it would require an enormous concentration of chemical energy to separate HCl from an alkaline component, it seemed reasonable to develop a theory of gastric acidity production which would not labor under this assumption of local alkali formation. A chemical reaction which seems particularly probable in this connection is the hydrolysis of an alkyl halide, with the production of hydrochloric acid and the corresponding alkyl alcohol. $RCl + HOH \rightarrow HCl + ROH$. The theory we have formulated involves (1) the mobilization of an organic chloride ester in gastric tissue at the time of gastric activity, and (2) the hydrolysis of this chloride ester, possibly under the influence of a specific enzyme activity. The alcohol thus formed may be reabsorbed at once or it may remain in the gastric juice, to be absorbed later in the intestine. The organic chloride is thought to be synthesized at the time of gastric activity, possibly in gastric tissue, as it is needed, from sodium chloride and the alcohol, with the simultaneous production of alkali—$NaCl+ROH \rightarrow RCl+NaOH$—thus accounting for the alkaline tide accompanying gastric secretion. To avoid an apparent contradiction, let it be explained that the alkali production accompanying the synthesis of the ester, though simultaneous with the acidity production, is in no sense coincident with it. The two reactions probably take place at two remote places, and certainly do not occur in the same place. The feature of this theory is that it permits of the formation of any concentration and amount of hydrochloric acid from *neutral* reactants and with a neutral product.

The existence of enzymes in gastric and other tissues specific for the hydrolysis of chloride esters has been demonstrated and reported on in a preliminary report.[1]

The results on the search for organic chlorides in gastric and other tissues may be summarized thus:

(1) A standard method for the differential fractional extraction of inorganic (ionized) and organic (unionized) chlorides from tissues has been developed.

(2) It has been shown that the fraction of organic chloride in some common tissues is very considerable, amounting to 10 to 50 per cent of all the chloride present. Thus 500 g. of wet fundus mucosa (100 g. dried tissue) contains 0.7 g. total chloride of which 0.35 g. is organic chloride.

(3) The behavior of the organic chloride in aqueous solution has been studied. It was found to be hydrolyzed with the production of both acidity and ionized chloride. The production of the acidity and of the ionized chloride do not always run parallel, but the discrepancies are probably due to an insufficient degree of purity of material.

(4) The distribution of this organic chloride in five different tissues has been studied. The fundus mucosa contains almost twice as much organic chloride per given weight of dry tissue as do the other tissues studied. The following table gives the results for 100 g. of dry tissue in any case.

TABLE I.

	Total Chloride	Organic Chloride
Fundus	.65— .75	.29—.31
Pylorus	.50— .65	.18—.21
Intestine	.45— .55	.14—.18
Liver	.30— .35	.15—.16
Blood	.9—1.0	.09—.13

(5) A comparative study of the distribution of different types of chloride in the tissues of fed and starved dogs showed no significant variations.

(6) These results, it is thought, furnish strong support for the theory that the hydrolysis of organic chlorides plays an important rôle in gastric acidity production.

This is a preliminary report.

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[1] Hanke, Martin E., *J. Biol. Chem.*, 1926, lxvii, 11-13.

3473

Postural Apnea in the Duck.

THEODORE KOPPANYI AND NATHANIEL KLEITMAN.

From the Department of Physiology of the University of Chicago.

Aside from the apnea which is produced in the duck by submergence or by pouring water over its nostrils, one can also obtain a complete cessation of respiration when the duck is held in the air in certain definite positions. This applies especially to the position of the head in space. We fully confirmed Huxley[1, 2, 3] and Paton[4] in that whenever the vertex of the head is directed downward, whether the body is in the prone or supine position, apnea ensues. Like Huxley, we observed a very marked slowing of the heart accompanying apnea.

If the duck is placed on its back, the neck will be curved so that the head is held in the air in normal position with the beak directed towards the tail (labyrinthine righting reflexes on the head). Under these conditions respiration is normal. When the head is bent backward so that the vertex is directed downward, respiration stops and the apnea continues as long as the forced position of the head is maintained. Occasionally the animal struggles for a moment or two and it appeared as if it might make some respiratory movements during the struggle. By connecting the head of the duck with the

Benedict portable respiration apparatus, we convinced ourselves that these movements usually do not involve any respiration. Occasionally they are accompanied by expiratory movements, and more rarely by inspiratory movements. Whenever there is a respiratory movement, it is always followed by a temporary acceleration of the slowed heart. These occasional respirations apparently suffice to keep the duck alive under these circumstances and some animals were subjected to incomplete postural apnea of as long as 30 minutes' duration. However, 2 of our animals succumbed to postural apnea, death being preceded by a few terminal expiratory movements. We consider this phenomenon of importance, in that it shows that reflex inhibition of respiration may be stronger than the chemical stimulation by the venous blood.

Huxley considered postural apnea as a reinforcement of apnea produced by submergence. In our experiments in submerging a duck in the prone and supine position in a large glass aquarium, we never saw the head assume the position required for postural apnea (vertex downward). On the contrary, the head was always held in the normal position and the apnea which was produced can only be due to the wetting of the nostrils. We conclude, therefore, that postural apnea is phenomenon *sui generis,* independent of diving.

Some ducks are refractive as regards the elicitation of postural apnea. That is not the result of the conditions of the experiment. If a duck shows postural apnea it shows it at all times. We are now studying various breeds of ducks, because we have some evidence that this reflex apnea is characteristic of certain breeds only. This is a preliminary report.

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[1] Huxley, F. M., *Quar. J. Exp. Physiol.*, 1913, vi, 147.

[2] Huxley, F. M., *ibid.*, 1913, vi, 159.

[3] Huxley, F. M., *ibid.*, 1913, vi, 183.

[4] Paton, D. Noel., *ibid.*, 1913, vi, 197.

3474

The Regulation of the Flow of Bile: III. The Rôle of the Gall Bladder.

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From the Hull Physiological Laboratory of the University of Chicago.

It has been demonstrated that the resistance to bile flow lies in the tenacity of the duodenum, rather than in the activity of a separate sphincter at the orifice of the common duct.[1, 2] When the duodenum is in a normal state of tonus, the secretory pressure of the liver causes the bile to be stored in the gall bladder under pressure. Besides the intra gall bladder pressure caused by elasticity of the organ, it has been claimed that there is forceful contraction of its musculature in expulsion of bile.[3, 4, 5, 6] Hendrickson[7] has shown that the smooth muscle tissue of the gall bladder of man and dog is relatively sparse, and not arranged in coats, as we might expect of an organ possessing marked powers of contraction. However, if the gall bladder possesses strong capabilities of contraction, it should, when in such a balanced physiological state as to display tonus rhythm, respond to one of the following means of excitation: activity of the duodenum; hormone action; stretching, as when distended by bile; drugs that act either directly or indirectly upon smooth muscle.

In dogs under light anesthesia with a small rubber balloon in the gall bladder connected with a water manometer, normal rhythmical tonus changes were recorded. No relationship between these and activity of the duodenum was found to exist. Pilocarpine and eserin tended to increase the tonus while adrenalin and atropine had the opposite effect. Barium chloride produced definite augmentation of the tonus rhythm. No strong contractions were produced by drug action.

A fat meal never brought about contraction in cat or dog, even when the gall bladder was distended. Following such a meal the bile pressure is increased by hepatic secretion. When the pressure is sufficient to overcome the resistance of the duodenum, bile flows. Fats, by lowering the tonus of the intestine, lower this resistance. It would seem that the bile flow reported by Boyden[3] and Whitaker[4] in cat and man as due to active contraction of the gall bladder, might be better interpreted as due to peristalsis and lowered tonus of the duodenum.

Edema of the gall bladder developed in approximately 25 per cent of the dogs. Its onset was always characterized by a gradually increasing pressure with decreasing respiratory oscillations and the absence of rhythmical tonus changes. The pressure began to increase during the first hour, and reached a maximum only after 2 or more hours. Because of the similarity between this rise of pressure in the gall bladder, and that described by Higgins and Mann[5] as due to a contraction, and because of the fact that a physiological contraction of this nature is not known, it seems that they may have encountered edema.

The contractions reported by McMaster and Elman[6] as being efficient in expulsion of bile represent no greater volume change in the gall bladder than takes place in strong tonus rhythm. It is doubtful whether rhythmical tonus contractions can play any part in the expulsion of bile, since they disappear when the pressure in the gall bladder rises to the height necessary to overcome resistance at the duodenum.

These experiments, which will be published in detail in the *American Journal of Physiology,* seem to warrant the conclusion that the gall bladder is incapable of contractions that might be construed as being of major importance in the flow of bile. This is a preliminary report.

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[1] Burget, G. E., *Am. J. Physiol.*, 1926, lxxix, 130.

[2] Copher, G. H., and Kodama, S., *Arch. Int. Med.*, 1926, xxxviii, 647.

[3] Boyden, E. A., *Anat. Rec.*, 1926, xxxiii, 201.

[4] Whitaker, L. R., *Am. J. Physiol.*, 1926, lxxviii, 78.

[5] Higgins, G. M., and Mann, F. C., *Am. J. Physiol.*, 1926, lxxviii, 339.

[6] McMaster, P. D., and Elman, R., *J. Exp. Med.*, 1926, xliv, 173.

[7] Hendrickson, W. F., *Johns Hopkins Hosp. Bull.*, 1898, ix, 221.

3475

What Causes the Psychic Secretion of Saliva in the Dog?

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From the Department of Physiology of the University of Chicago.

It is a well known fact that a dog, when hungry, will salivate in the presence of food. Pavlov called it a natural conditioned reflex, developed in the animal as it learns to recognize certain matter as food. But is this salivation caused by the sight or the odor of the food? To answer this question I employed a number of dogs with fistulae of the submaxillary glands. These dogs were starved for a couple of days and then placed into a stand and a collecting tube attached to them in the manner described in another communication.[1] They were allowed to remain in the stand for varying lengths of time, as a control. In 30 to 60 minutes they generally secrete one or two drops of saliva. A perfectly transparent glass museum jar of prismatic shape was partly filled with cooked bread and meat, the fare that the dogs subsisted on for a year. The flat ground edges were covered with a slab of plate glass and sealed by means of vaseline.

The jar was placed in front of the animals at a distance of 2 to 3 inches from its snout. In another half hour there was no secretion of saliva. If at the end of this period the cover was taken off the jar, the animal immediately changed its indifferent attitude, became agitated, began to sniff and to stretch its neck toward the food. Saliva began to flow through the fistula and drip from the mouth at once. In 15 minutes it generally secreted from 0.5 to 2.5 cc. of saliva from the fistula alone. Since all these animals were accustomed to the appearance of the food, it is clear that the dog does not recognize food at sight, and that the natural conditioned reflex or psychic flow of saliva is due to the odor of the food only.

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[1] Kleitman, N., and Crisler, G., *Am. J. Physiol.*, 1927, lxxiv, 571.

3476

Effect of Intraperitoneal Magnesium Sulphate on Parathyroidectomized Rats.

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From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Having been engaged in an experiment which was hampered by the high death rate among thyroparathyroidectomized rats, the authors have developed an exceedingly simple and convenient method for reducing this mortality. This method was suggested by the work of Luckhardt, Waud, and Brannon[1] on the effects of magnesium chloride given *per os* to dogs.. The method consists in the intraperitoneal injection of magnesium sulphate. For a rat weighing 150 grams the injection consists of 5 cc. of a 1 per cent solution of the crystalline salt. Experience has shown that asepsis is unnecessary here, so that the dosage can be given to any number of rats with greater speed and accuracy than would be possible by oral administration.

A normal rat can easily tolerate as many as 5 hourly injections of this quantity. After each injection one sees the manifestations of the well-known anesthetic and curare-like actions of magnesium. The rat becomes unable to raise its body off the floor of the cage, and may cease to right itself when laid on its back. If, instead of the divided doses, one gives a single dose of 25 cc., the above symptoms are followed by death.

Parathyroidectomized rats should be watched closely beginning, say, with the tenth or twelfth hour after operation. When the tetany has become sufficiently marked to prove the completeness of parathyroid extirpation, the injection is given. No signs of pain or irritation follow; inactivity, be it due to apathy or myasthenia, sometimes appears within 2 minutes. The signs of tetany, however, seem to persist a few minutes longer; carpopedal spasms, or the characteristic fibrillary activity of the vibrissae, may continue for a time after the rat has become unable to walk. Recovery from the immobilizing effects of the magnesium occurs within an hour, but the freedom from tetany lasts much longer. At first daily injections are necessary, given preferably in the evening, so as to preclude possible attacks of tetany during the night; in less than a week (the rat having survived the stage of acute deficiency) the injections need no longer be given daily.

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[1] **Luckhardt, Waud, and Brannon, *Am. J. Physiol.*, 1926, lxxvi, 228.**

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3477

Effect of Venostasis on Heart Failure with Particular Reference to Cardiac Asthma.

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Though the etiology of cardiac asthma is unknown, a markedly increased cardiac output and blood velocity throughout the body and especially the lungs are according to Eppinger, Papp and Schwartz[1] significant factors. They explain the attack by a relatively left ventricular insufficiency dependent on the excess of blood brought to the lungs and heart. In view of the convincing nature of their experiments the writer has attempted to check the circulatory overflow in the periphery by the compression of the veins in the four extremities. This is conveniently performed by applying four blood pressure cuffs around the extremities as near to the trunk as possible. These cuffs are all in series and connect with an ordinary blood pressure manometer and rubber bulb. We have called the apparatus the "Venostat." (Fig. 1.)

The cuffs are gradually inflated up to diastolic pressure and maintained at this level for 10 to 12 minutes. They are then very gradually deflated.

The application of this procedure during a cardio-asthmatic attack relieves the symptoms in about 5 minutes, and frequently terminates the attack by the time the treatment has been completed. Objectively sonorous and sibilant rales that may be present over the lungs become very faint or disappear entirely.

During the treatment the extremities become engorged due to compression of the veins. The arteries remain patent. A considerable volume of the blood now collects in all the four extremities.

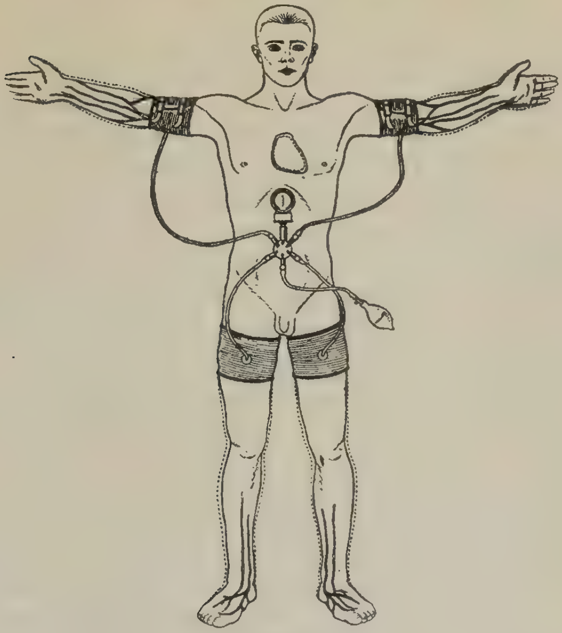

FIG. 1.

The cardiac outline becomes slightly reduced on the X-ray plate. Even the slight diminution in the cardiac silhouette as shown recently corresponds with great changes in the volume of the heart. Thus the heart and lungs are temporarily unloaded. When one recalls the fact that both of these organs are never completely at rest during life—even this partial rest for a relative short time may be sufficient for the heart to recuperate. The possibility that a reflex effect on the heart or the respiration, due to the stasis of blood in the extremities, may also be operative, is not to be denied.

In the belief that venostasis unloads the heart sufficiently for recuperation, its effect was tried on clinical cases of heart failure requiring large doses of digitalis. Only far advanced cases were studied. Digitalis was not given and no other treatment besides the restriction of salt and fluid intake was applied. Venostasis was produced for 10 to 12 minutes every two hours. Marked improvement in the dyspnea resulted. Orthopnea disappeared. The edema lessened and the water excretion increased—in some cases exceeded the fluid intake. After a week, however, digitalis had to be given again because of the return of the edema. These were far advanced cases, however. We were thus led to believe that venostasis is capable of disembarrassing an insufficient heart; only the far advanced cases must, in addition, be given digitalis.

Our results are more intelligible in the light of the experiments of Harrison and Leonard,[2] who have found that digitalis acts by reducing the cardiac output. From their work it appears that digitalis is a cardiac sedative rather than a stimulant. Eppinger observed a case of heart failure with a minute output of 10 liters that was reduced to 5 liters when compensation was restored. The combined findings of these investigators may modify our views on circulatory failure where a reduction of cardiac output was hitherto assumed to exist and treatment was directed towards increasing the systolic discharge. If further study should permit general application of these principles to the conception and treatment of cardiovascular failure, the keynote of therapy may be cardio-sedative measures. It appears to us that venostasis as we apply it, fulfills this requirement.

The difference between the action of venostasis and digitalis is that in the latter the diastolic filling is reduced by an increase of cardiac tonus, while in venostasis the shunting of a considerable volume of blood in the extremities by simple mechanical means partially depletes the heart and accomplishes the same purpose.

Conclusion: Venostasis is an effective procedure in cardiac asthma and heart failure. It can frequently replace digitalis in the treatment of cardiac decompensation. A convenient apparatus called the "Venostat" is presented.

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[1] Eppinger, H., Papp and Schwartz, Asthma Cardiale, Julius Springer, Berlin, 1924.

[2] Harrison, T. R., and Leonard, B. W., *J. Clin. Investigation*, 1926, iii, 1.

3478

Morphological and Chemical Studies of the Blood in Chronic Duodenal Obstruction.

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*From the Department of Pathology, College of Physicians and Surgeons, Columbia University.**

In the following report we wish to describe our observations of the blood changes in dogs with experimentally produced chronic duodenal obstruction. In the 12 dogs which were studied, the obstruction existed for periods extending from 2 months to 13 months.

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* This work was aided by a grant from the Eli Lilly Company.

In previous communications[1] we described the changes in motility, the marked stasis and the great increase in intestinal bacteria in the duodenum, above the site of the obstruction. The types of organisms were those usually encountered in the intestine of dogs, *i. e., B. coli, Enterococci* and *B. welchii.* The influence of this prolonged stasis upon the blood was determined by frequent hemoglobin determinations, red and white cell counts, differential counts, and chemical analyses. In addition, gastric analyses were made. especially for the presence of free HCl.

All of the dogs developed a secondary anemia of variable intensity. The maximum drop in red cells ranged between one million and two and one-half million. The hemoglobin index was always below one. There were frequent remissions during which the red cell count was normal. The leucocyte count was too irregular to be significant. The morphology of the erythrocytes remained practically unaltered; occasionally a few normoblasts and a slight anisocytosis were observed in the smears. Free HCl was always present in the gastric contents.

The chemical examination of the blood included urea nitrogen, chlorides and carbon dioxide combining power. Examinations were made when the dogs were free of any symptoms. Figures which were obtained during or after periods of vomiting or shortly before death were excluded. The only significant finding was an increase in urea nitrogen. However, the variations in the urea nitrogen of a series of normal dogs observed over a prolonged period were approximately the same as those with duodenal obstruction.[2] It was noted that an increase in urea nitrogen occurred more often in dogs which subsequently showed kidney lesions.

In view of the work done by Seyderhelm, Löwenberg, Cornell, Torrey and others, with *B. coli, B. welchii,* and their toxins, we thought it possible that an anemia of the pernicious anemia type might be produced. The changes we observed were characteristic of the secondary anemias. However, in pernicious anemia, achylia is present, whereas in our experimental animals free HCl was always found in the gastric contents. Further experiments will be directed toward neutralizing or eliminating the gastric HCl in dogs with chronic duodenal obstruction.

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[1] Berg, B. N., Meleney, F. L., and Jobling, J. W., *Arch. of Surg.*, 1927, xiv, 752.

[2] McKinley, E. B., (to be published later).

3479

Toxicity of Filtrates of Staphylococcus Aureus From Human Infections and From Normal Nasopharynx.

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In 1899 Von Lingelscheim[1] observed necrosis in the skin of rabbits following the intracutaneous injection of staphylococcus filtrates. Neisser and Wechsberk[2] repeated the original experiments of Lingelscheim and studied the toxic properties of the filtrates more fully. They demonstrated both active and passive immunity in rabbits and found the toxic qualities of the filtrate to be extremely thermolabile. The filtrates no longer gave cutaneous reactions after heating at 56° C. More recently Parker[3] and her associates have carried out experimental work along the same line. They find that filtrates of staphylococcus grown under proper conditions cause necrosis in the skin of rabbits. They obtain antitoxic serum in rabbits and horses. This antitoxin prevents the necrosis in the rabbit, and neutralizes staphylococcus filtrate, according to the law of multiples. In their first experiments few of the strains with which they were working gave toxic filtrates. Later filtrates from 13 or 14 strains gave cutaneous reactions neutralized by a common antitoxic serum.

When filtrates of bacteria give cutaneous reactions of this type there is a possibility that the toxicity of the filtrates may be associated with the virulence of various strains. We have studied 31 strains of *Staphylococcus aureus* with this possibility in view. The strains selected were all hemolytic. They were obtained from two sources. First, from infectious processes including furuncles, abscesses, acute staphylococcus anginas and from the blood stream in cases of septicaemia, and second from the nasopharynx of normal individuals who were carriers of staphylococcus. The first subculture of each strain was grown in 4 per cent proteose peptone broth for 4 days in an atmosphere of carbon dioxide. Parker[3] has found this method especially suitable for the production of toxin. The sterile filtrates were then tested in the skin of rabbits to determine the minimum reacting dose, control experiments were carried out with filtrates boiled for 10 minutes, and neutralization tests made with antitoxic horse and rabbit sera. The horse serum was prepared by Parker.

TABLE I.

Comparison of the toxicity of Staphylococcus aureus filtrates and neutralization reactions with antitoxic sera.

Source	Cutaneous reactions in rabbits					Boiled filtrate	Normal rabbit serum	Rabbit antitoxin	Horse antitoxin
	1 cc.	.1 cc.	.05 cc.	.01 cc.	.005 cc.				
Abscess	+	+	+	+	—	—	+	—	—
Furuncle	+	+	+	+	—	—	+	—	—
Furuncle	+	+	—	—	*	—	+	—	—
Furuncle	+	+	+	—	*	—	*	*	—
Furuncle	+	+	+	—	*	—	*	*	—
Furuncle	+	+	+	—	*	—	+	—	—
Furuncle	+	+	+	—	*	—	+	—	*
Furuncle	+	+	—	—	*	—	*	*	—
Furuncle	+	+	+	—	*	—	+	—	—
Furuncle	+	+	+	+	±	—	+	—	—
Bloodstream	+	+	+	—	*	—	*	*	—
Furuncle	+	+	+	—	*	—	*	*	—
Abscess	+	+	+	+	—	—	*	*	—
Angina	+	+	+	+	±	—	+	—	—
Furuncle	+	+	+	±	—	—	+	—	*
Bloodstream	*	+	+	±	—	—	*	—	—
Pharynx	+	+	+	+	—	—	+	—	—
Pharynx	+	+	+	±	—	—	*	*	—
Pharynx	+	+	+	+	+	—	+	—	—
Pharynx	+	+	+	+	±	—	+	—	—
Pharynx	+	+	+	+	—	—	+	—	—
Pharynx	+	+	+	+	+	—	*	*	—
Nose	—	—	—	*	*	*	*	*	*
Nose	+	+	+	—	*	—	*	*	—
Nose	—	—	*	*	*	*	*	*	*
Nose	+	+	—	—	*	—	+	—	—
Nose	+	+	+	—	*	—	*	*	—
Nose	+	+	+	—	*	—	+	—	—
Nose	—	—	*	*	*	—	*	*	*
Pharynx	+	+	—	—	*	—	*	*	—
Pharynx	*	+	+	+	+	—	*	*	—

* Reaction not done.

The data have been arranged in the accompanying table. The strains have been placed in two groups. The first includes strains from the infections and the second those from normal carriers. After each strain the reaction obtained in rabbits with decreasing amounts of filtrate has been indicated in the appropriate column. The doses employed were 1 cc., 0.1 cc., 0.05 cc., 0.01 cc., and 0.005 cc. of filtrate, The results of neutralization with antitoxic sera, normal rabbit serum, and of thermolability have been indicated in additional columns.

An analysis of this table shows that the toxins produced by the various strains of *Staphylococcus aureus* which we have studied are apparently identical, since the reaction obtained with each filtrate could be prevented with the same antitoxic sera in all instances, and the toxic properties of all the filtrates had the same degree of thermolability. This applies to strains from both groups studied. Among the strains obtained from the acute infections there appears to be no relationship between the severity of the infection clinically, and the ability of the corresponding strain to produce toxin. When the two groups are compared in regard to toxin production there is no significant difference. The only noteworthy feature is the fact that all the strains from infections produced toxin, while three of the strains from the nasopharynx either produced none, or produced such small amounts that no reaction followed the intracutaneous injection of filtrate.

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[1] Von Lingelscheim, W., *Beitr. z. Exp. Ther.*, 1899, i, 49.

[2] Neisser, M., Kolle and Wasserman, *Handbuch der Pathogenen Mikroorganismen*, Jena, 2nd Edition, 1912, iv, 375.

[3] Parker, J. T., *J. Exp. Med.*, 1924, xl, 761; Parker, J. T., Hopkins, J. G., and Gunther, A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 344; Parker, J. T., and Banzhof, E. J., *J. Immunol.*, 1926, xiii, 25.

3480

Filtration Experiments With Bacteriophage Employing a Physiological Filter-Pia, Dura and Arachnoid.

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Much has been written concerning filtration experiments with various types of artificial filters such as the Berkefeld, Chamberlain and collodion membranes. Recently a new type of filter has come upon the market, the Seitz-Werke filter, and for certain types of work is apparently quite efficient. The efficiency of all these types of artificial filters, as far as the mere removal of bacteria is concerned, is beyond question. It is only when investigators attempt to draw fine conclusions from filtration experiments employing such filters that their value is questioned. A substance, for example, which is filterable through a Berkefeld Type N or W filter in the hands of one investigator may not be filterable when presumably the same type of filter in every respect is employed by another. Such results are very confusing and often discouraging. This has been particularly true in the field of filterable viruses and indeed, there are many viruses so classified at the present time upon the sole basis that they have, in the hands of some workers, been able to pass through one of these artificial filters. In the first place it is well known that filters of this type, even when new and unused, though prepared by standard methods, still vary one from the other. No two are precisely the same. Again the amount of pressure employed by different workers against these filters is never the same and further in many instances the filter is not controlled. The time element of filtration is known to be important and there are always the variations in individual technique which add to the general unreliability of results. These criticisms are well known to all laboratory workers and as a result we have come to regard filtrability through such filters in the same sense as diffusibility, as only a relative concept.

Upon the basis of such methods, then, we have come to classify what we regard as the etiological agents of a host of diseases affecting man, animals, insects and plants. The term "filterable" has become general in use and based purely upon the methods employed, is becoming more and more burdensome. The great temptation at present is to consider all those diseases in which the causative agent is unknown as disease due to a "filterable virus." Taken together with our general lack of definite information regarding the nature of these agents, we appear to have adopted a term, perhaps because of its convenience largely, which is exceedingly indefinite.

In the present communication experiments are described concerning the filtration of bacteriophage from the blood stream to the spinal fluid and from the spinal fluid to the blood stream in rabbits. Conceiving of the pia, dura and arachnoid as a physiological filter system one should be able here to think of the filter as representing uniform conditions as near as is possible.

It is well known that physiological saline solution is absorbed rapidly when injected into the sub-arachnoid space even in large quantities. In several diseases therapeutic serum is administered by this route with beneficial results. The substances introduced into the sub-arachnoid space have been limited largely to these two as untoward results have followed when certain dyes and drugs have been administered this way.[1] It is also well known that the amount of therapeutic immune serum, drugs (such as salvarsan) and certain dyestuffs, reaching the spinal fluid when injected intravenously, is comparatively small, if any at all. According to the physiologists the cerebrospinal fluid may be considered a dialysate from the blood or an ultra-filtrate. Its specific gravity ranges from 1.007 to 1.008 while that of blood varies from 1.041 to 1.075. When freshly drawn the pH of cerebrospinal fluid is 7.4 which is approximately that of fresh blood. Both blood and spinal fluid become more alkaline on standing. According to some authors the cerebrospinal fluid is quite similar to blood in many other respects. It contains from 17 to 26 mg. of non-protein nitrogen, 7 to 13.5 mg. of urea nitrogen, .07 to 0.1 per cent of dextrose, and .02 per cent of protein, all based on 100 cc. The trace of protein is globulin in nature, it contains no albumin or fibrinogen but there are traces of other organic substances to be found in it. Hewett[4] in a recent paper concludes that in normal cerebrospinal fluid there is about .02 per cent albumin and about .003 per cent globulin, of which by far the greater part is pseudoglobulin. While it appears fundamentally that most of the constituents of the cerebrospinal fluid come from the blood stream which carries nutrition to the tissues of the body, it is evident that some of the blood elements are unable to pass through the filtration apparatus, notably the protein aggregates. At least they do not appear there in any great quantities and some of them not at all (albumin and fibrinogen). The explanation for this phenomenon might explain why drugs, certain dyes and immune substances fail to pass since it is possible they may become compounded with blood proteins when injected intravenously. Flateau[3] has recently been able to pass fuchsin from the blood stream of rabbits to the spinal fluid by first injecting various substances, such as atropine or glucose solution intravenously or intraspinously. Whether the actual permeability of the membranes is altered in this way is still questionable, however. Le Fèvre[5] has shown that certain dyes, such as methylene blue, trypan blue, neutral red and others exert a favorable influence upon the fixation by the central nervous system of certain neurotropic viruses such as the virus of herpes and of rabies, presumably by acting on the vascular endothelium of the meninges.

Intraspinous Injection of Bacteriophage.

3 cc. Coli "D" bacteriophage in broth pH 7.6 were injected intraspinously into a rabbit. Two hours and 24 hours later the blood, feces, urine and spinal fluid were tested for the presence of bacteriophage. The bacteriophage was active in a 1:10,000,000 dilution.[2] The first concentrated serum cerebro-spinal fluid and urine were tested for bacteriophage by the test tube method and were found to be negative. This does not prove conclusively that the bacteriophage is absent, since we have shown elsewhere that due to the particulate nature of the bacteriophage in great dilutions that the test tube method is unreliable as a test for its presence or absence.[2] Other fractions of the serum, cerebro-spinal fluid, urine and feces were added to broth and incubated with the susceptible Coli "D" culture and repeated sub-passages were then tested.

TABLE I.

Material Tested	1/23	1/24	1/25	1/26	1/27	1/28	1/29
Conc. serum	—	*	*	*	*	*	*
Passage serum	*	—	∓	+	+	+	+
Passage feces	*	—	—	—	—	—	—
Conc. urine	—	—	*	*	*	*	*
Passage urine	*	—	—	—	—	—	—
Conc. cerebro-spinal fluid	—	*	*	*	*	*	*
Cerebro-spinal passage fluid	*	—	∓	+	+	+	+
Control bacteriophage	+	+	+	+	+	+	+

* Not determined.

It will be noted in Table I that the serum from blood removed two hours after intraspinous injection of bacteriophage became questionably positive after the second passage with the sensitive strain while it was definitely positive after the third passage. The same was true for the cerebro-spinal fluid. The feces or urine never became positive, even after five passages with the sensitive strain. The control was, of course, positive throughout the experiment. This experiment serves to demonstrate that a substance such as bacteriophage which can be detected in high dilutions is capable of passing from the cerebro-spinal fluid to the blood stream. This we might expect since the normal direction of flow is in this direction.

In a second experiment the blood serum, cerebro-spinal fluid, urine and feces of a normal rabbit were first tested for bacteriophage against the sensitive B. Coli "D". They were found to be negative. The animal was then given 5 cc. intravenously of B. Coli "D" bacteriophage active in a dilution of 1:10,000,000. The bacteriophage could not be demonstrated in the spinal fluid, feces or urine after seven passages with the sensitive strain though it was present in the blood serum in the third passage. The control bacteriophage remained positive during each passage.

These experiments indicate that a particular substance such as the bacteriophage is filterable from the cerebro-spinal fluid to the blood stream in rabbits but not from the blood stream to the cerebrospinal fluid. Our failure to detect the bacteriophage in the urine and feces is not conclusive evidence that it is not excreted through both of these channels, since the factor of dilution in both instances is quite different from that of the blood and cerebrospinal fluid.

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[1] McKinley, E. B., and Holden, M., *J. Am. Med. Assn.*, in press.

[2] McKinley, E. B., and Holden, M., *J. Infect. Dis.*, 19??, xxxix, 451-456.

[3] Flateau, E., *Revue Neurologique*, 19??, xxxiii, 281-360.

[4] Hewett, L. F., *Brit. J. Exp. Path.*, viii, 1, 84-92.

[5] Le Fèvre, M. de A., *Comp. rend. de la Soc. de Biol.*, t, xcvi, 206-207.

3481

Liver Injury in Acute Alcoholic Poisoning.

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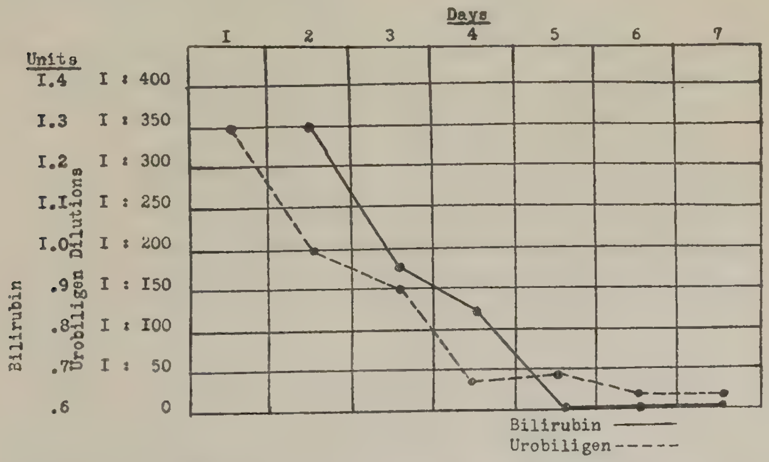
Although in most text books of Pharmacology and Medicine the statement is made that alcohol has a directly injurious effect on the liver, recently much doubt has been expressed as to an etiological relationship between alcohol and liver disease. An opportunity of studying altered liver function in acute alcoholic poisoning has been taken advantage of in an attempt to throw light on this question, and a short statement of the positive results obtained is given.

The subjects of the observations were 17 of the patients admitted to Bellevue Hospital with the diagnosis of acute alcoholic poisoning. Two tests of liver function were employed, namely the indirect Van den Bergh quantitative test for bilirubin and the quantitative determination of urobiligen[1] in the urine. The blood and urine specimens were obtained at arbitrary intervals over a period of several days.

In all 17 cases there was found an increase in the amount of bilirubin in the blood serum. Accepting as normal .6 to .8 unit, the increase found ranged from .85 to 2.1 units and all except one were 1 unit or above. The increase persisted for four or five days with a gradual decline to the normal. In 5 cases urobiligen estimations were made. An increase was found ranging in dilutions from 1:90 to 1:350. The urobiligen curve closely corresponded to the bilirubin curve. This increase of both bilirubin and urobiligen was directly proportionate to the severity of the poisoning.

A typical chart is presented.



It may be concluded from these observations that in acute alcoholic poisoning there is definite evidence of liver injury and this is proportionate to the severity of the poisoning.

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[1] Wallace, G. B., and Diamond, *Arch. Int. Med.*, 1925.

3482

Effect of Antipneumococcus Sera on Leucocytes in vitro and on the Agglutination of the Red Blood Cells.

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(Introduced by Wm. H. Park.)

From the Littauer Pneumonia Fund of New York University and the Medical Service of Harlem Hospital.

In the specific antibody pneumococcic horse sera (Felton[1] and Banzhaf[2]) heteroleucotoxins for certain human white cells, analogous in action to the isoleucotoxin of Doan,[3] were found, though never in titre strengths exceeding 1 in 18. Unfortunately, these observations invalidate this *in vitro* procedure as a probable cause of, or test upon which to base prediction of chill reactions in the serum treatment of pneumonia cases.

The incidental observation that a chill producing serum did agglutinate the red cells *in vitro,* whereas a non-chill producing serum did not produce such heteroagglutination of the human red cells, induced us to restudy this phase of the question. Our studies confirm those of Kolmer and Matsumoto (1920) in that the *in vitro* agglutinations have been found to bear no constant relationship to serum reactions. The small amounts of slowly injected serum apparently have no appreciable effect on the circulating red blood cells.

The distribution of blood groups in 100 cases of pneumonia was observed and showed the usual distribution.

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* With technical assistance from Miss Mabel Chatfield.

[1] Felton, L. B., *Boston Med. and Surg. J.*, 1924, cxc, 819.

[2] Banzhaf, E. J., *Johns Hopkins Hosp. Bull.*, 1911, xxii, 241.

[3] Doan, C. A., *J. Am. Med. Assn.*, 1926, lxxxvi, 1593.

[4] Kolmer, J. A., Matsumoto, M., *J. Immunol.*, 1920, v, 75.

3483

A New Agglutinable Factor Differentiating Individual Human Bloods.

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By absorbing a number of anti-human blood immune sera from rabbits with the blood corpuscles of certain individuals regardless of the group, fluids were obtained from a few sera which give a

TABLE I.

Bloods	Group I.				Group II.				Group III.				Group IV.			
	J. K.	S. A.	D. F.	L. S.	W. F.	W. D.	P. L.	A. R.	E. J.	A. J.	M. R.	S. M.	A. S.	H. S.	F. C.	H. G.
Serum No. 1020 diluted 1:20, absorbed with human blood, P. L. (Group II.)	0	++	+++	++	++	+++	0	++	0	+++	++	+	++	0	++	+++

Technic: 2 gtts. absorbed serum.
1 gtt. saline.
1 gtt. 2.5 per cent blood suspension.

TABLE II.

	Group I.		Group II.		Group III.		Group IV.		Total number	Total positive	Total negative
	+	0	+	0	+	0	+	0			
White	14	2	20	3	15	4	4	2	64	53	11
Colored	15	5	12	5	10	4	1	0	52	36	16

sharp differentiation of individual human bloods within the common blood groups.

Among 116 individuals selected from the four blood groups the distribution of the agglutinable factor (which may be designated as M) was as shown in Table II.

This reaction is distinguished by its intensity from some others known to show individual differences within the groups, such as the reactions with cold agglutinins.

This is a preliminary report.

3484

Further Observations on the Extraction of Precipitable Substances of Bacilli.

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From the Laboratories of The Rockefeller Institute for Medical Research.

In continuation of the work reported in a previous note[1] the following results were obtained:

The precipitable substances extracted by dilute alcohol from various bacilli are of different sorts. While the substance derived from *B. typhosus* loses its activity by digestion with trypsin, the extracts of *B. paratyphosus* B and *B. proteus* X19 resist tryptic digestion like that of *V. cholera*. The substance extracted from *B. paratyphosus* B and *B. proteus* yielded reducing sugar after hydrolysis. The N content of the substance obtained from *B. typhosus* approximates that of proteins.

After treating *B. typhosus* several times with hot 75 per cent alcohol, an extract was prepared by heating with saline solution. This substance differed serologically from the above mentioned substance of typhoid bacilli, in that it was precipitated strongly by immune sera prepared with typhoid bacilli extracted with dilute alcohol, and gave weak reactions with immune sera prepared with dilute alcohol extracts. It is likewise digested by trypsin, and therefore both products apparently differ from that described by Douglas and Fleming.[2]

After digesting with trypsin, the precipitable substance extracted with dilute alcohol from *B. paratyphosus* B still reacted with *B. paratyphosus* B immune sera, but not with immune sera prepared with the dilute alcohol extract of *B. typhosus* or *B. enteridis*. This crude preparation of *B. paratyphosus* B produced strongly agglutinating and precipitating sera when injected into rabbits.

A second extract was obtained also from *B. proteus* HX19 with saline solution from the bacilli previously treated with dilute alcohol. In contrast to the dilute alcohol extract this preparation had a high N content and was digested by trypsin. Both the dilute alcohol and the saline extracts reacted with common HX19 and OX19 immune sera but gave practically no precipitations with typhoid and paratyphoid antisera. The relation of these substances to that described by Przesmycki[3] is being studied. This is a preliminary report.

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[1] Landsteiner, K., and Furth, J., Proc. Soc. Exp. Biol. and Med., 1927, xxiv, 379.

[2] Douglas, S. R., and Fleming, A., *Brit. J. Exp. Path.*, 1921, ii, 131, 175.

[3] Przesmycki, F., *Soc. Biol.*, 1926, xcv, 744.

3485

Antigenic Composition of the Watery Extracts of the Tubercle Bacillus.

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The differences found between the eggwhite hypersensitiveness of tuberculous guinea pigs and the tuberculin hypersensitiveness induced us to continue our former work[1] on the protein antigens of the tubercle bacillus. We publish this preliminary report because the detailed examination of the observations made requires considerable time. The following extracts of a virulent freshly cultivated strain of tubercle bacilli were examined in their relation to the antigen antibody reactions and the tuberculin reaction: Direct extract was obtained with distilled water. The growth on glycerin bouillon was washed without being broken up with distilled water, and filtered quickly, then suspended and slowly ground for two days in distilled water with the addition of some chloroform. If the culture is washed longer with distilled water and filtered slowly one obtains an extract of very slight efficiency and very little protein content. This was the reason that in our first publication, we overlooked the interesting properties of this extract. After being filtered and washed again with distilled water the bacillary mass was dried and extracted with ether (2 days) and alcohol (twice during 3 hours), then extracted twice with distilled water, being thoroughly ground (5 days) (extract after defatting), and six hours (including filtration) with 0.2 per cent NaOH (NaOH extract). With every extract several sera were obtained from tuberculous guinea pigs in the first weeks after the infection. The complement fixation, precipitation, and tuberculin effect of the extracts and different precipitates of them with HCl, alcohol, NaOH, etc., were examined. None of the sera has given complement fixation with the alcohol extract of the tubercle bacillus, or precipitation with the purified carbohydrate precipitable substance, but they gave strong precipitation with the HCl precipitates of the extracts.

With these sera and with rabbit sera obtained with tubercle bacillus emulsion, three different antigens were clearly distinguished in the extracts, and the presence of two more made probable. There is a distinct antigen present in the direct extract HCl precipitate, in the filtrate from the HCl precipitate of the same extract (beside the carbohydrate precipitable substance) and in the HCl precipitate of the NaOH extract. From the HCl precipitate of the extract, after defatting of the bacteria, it could not yet be decided whether it contains an independent antigen or whether it is a mixture of the others, and there is indication that the filtrate of the direct extract contains two antigens.

The differences in the reaction of the different preparations are marked. For instance the filtrate after HCl precipitation of the direct extract gives a complete reaction in the amount of .004 cc. with the corresponding serum, and in .12 cc. is negative with the serum of the NaOH extract. The HCl precipitate of the NaOH extract gives a complete reaction in .0005 cc. with its own sera, and only in .03 cc. with one serum obtained with the direct extract, and in .015 cc. with the other serum.

All extracts and all preparations obtained from them have tuberculin activity. From the extract after defatting, and from the NaOH extract, both the antigens for complement fixation and the substances with tuberculin effect are precipitated to a large extent with HCl together with the protein substances. From the direct extract about half of the tuberculin effect remains in the filtrate, after the precipitation with HCl. The precipitate which contains about 14.3 per cent N, after two precipitations and washing with acid-distilled water, contains only a small fraction of the original activity of the extract. The tuberculin effect and the antigenic effect in the complement fixation, demonstrable with the sera we have had at our disposal, are not bound to the same substance in the filtrate. The antigens are precipitated with the addition of 1 volume of alcohol, and the largest part of the tuberculin active substances are precipitated only with the addition of 5 to 9 volumes of alcohol. The properties of the direct extract filtrate are very similar to the properties of the residue material described by Zinsser,[2] and the effective substance may be similar to the water-soluble protein isolated by Long and Seibert[3] from the culture fluid of tubercle bacilli.

In the hypersensitiveness obtained with eggwhite there is present an independent sensitization against egg albumen and globulin. We tried in several ways to decide whether there is present a specificity of the different fractions of the bacillus in the tuberculin effect. Two preparations, the direct extract HCl filtrate and the NaOH extract HCl precipitate, were compared. Certain animals are more sensitive to repeated tests with one or the other of the preparations. In the first weeks after the infection the sensitiveness appears first toward the direct extract filtrate; sensitiveness toward the NaOH extract seems to develop later.

If tuberculous guinea pigs are injected subcutaneously with large doses of the NaOH precipitate, the sensitiveness toward the NaOH precipitate is much more diminished than the sensitiveness toward the direct extract filtrate. The subcutaneous injection of the direct extract filtrate, which causes more violent general reaction, lowers the sensitiveness considerably toward both preparations, but decidedly more towards the filtrate. The subcutaneous injection of NaOH precipitate causes usually a much larger infiltration than the injection of the filtrate. On the other hand, according to our present observations from solutions of equal strength in the skin test, much less is needed from the filtrate of direct extract to kill a tuberculous guinea pig than from the NaOH extract. These observations point to a specificity of the different fractions also in the tuberculin effect, but they might also be the result of the circumstance that the active group is in different binding in the different fractions of the bacillus. This is a preliminary report.

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[1] Dienes, L., Schoenheit, E. W., and Scheff, L. D., *Am. Rev. Tuber.*, 1925, xi, 151.

[2] Zinsser, H., *J. Exp. Med.*, 1921, xxxiv, 495.

[3] Seibert, Florence B., and Long, E. R., *Am. Rev. Tuber.*, 1926, xiii, 400.

3486

Local Sensitiveness of Tuberculous Guinea Pigs Against Egg White Similar to Arthus Phenomenon.

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Further experience with the local sensitiveness produced with eggwhite in tuberculous guinea pigs[1] revealed that this sensitiveness shows characteristics very similar to the characteristics of the Arthus phenomenon of rabbits, and it differs in essential characteristics from the tuberculin sensitiveness. The most important differences are:

1. The presence of precipitins in the blood serum. Although there is not a strict parallelism present between the hypersensitiveness and precipitins (we found a few exceptions to the rule) yet we were not able to separate them regularly.
2. The hypersensitive animals are not killed through the subcutaneous injection of quite large amounts of eggwhite although a very extensive necrosis develops on the site of the injection.
3. They are killed through the intravenous injection.

These differences are either the expression of a different physiological mechanism or they are the result of different properties of the antigens in the reaction. To examine the latter possibility we treated tuberculous guinea pigs with Ty bacilli and timothy pollen, supposing that their antigens are nearer the antigens of the tubercle bacilli than eggwhite. With Ty bacilli we were not able to obtain any hypersensitiveness. But as no increase of the agglutinins was present, compared with the controls, the conditions of the experiment were not optimal. With the timothy pollen in some cases we obtained pronounced hypersensitiveness in tuberculous animals, also increased precipitin formation, but as we obtained positive results in only a few cases we were not able to study the properties of this sensitiveness, so with this experiment we did not succeed in coming nearer to an understanding of the tuberculin sensitiveness.

This is a preliminary report.

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[1] Dienes, L., and Schoenheit, E. W., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 32.

3487

The Permeability of the Placenta of the White Rat to a Specific Hemolysin.

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In view of the controversy upon placental permeability, the following experiments were undertaken to keep the animal under observation for a longer period than has heretofore been done, and to test placental permeability by means of a definite indicator (in this case immune serum hemolysin against guinea pig corpuscles). The white rat was used as it has been shown (Nicholas, 1925,[1] 1926[2]) that this animal is capable of withstanding the shock incident to the necessary surgical procedure without subsequent abortion. Frequent injections could, therefore, be made into the foetus, and its condition followed through frequent observations.

Specific immune serum hemolysins were prepared by the following method: 3 cc. of a 50 per cent suspension of washed guinea pig corpuscles were injected intraperitoneally twice a week for 3 weeks; the animals were not injected during the succeeding 10 days. The second series of injections was begun at the end of this period, twice a week for 3 weeks, consisting of a 60 per cent corpuscle suspension. The percentage of the corpuscle suspension was increased 10 per cent each week. The serum of 12 rats so treated was then pooled, and the titer taken. While the rat is regarded as being a poor animal, from the standpoint of the strength of the reaction obtained, the low titer hemolysin (titer 500) secured had the advantage of being nontoxic to the rat foetuses.

The experiments with the above material are: 1. The injection of immune serum hemolysin into 14 to 19 day old foetuses; 2. The injection of 50 per cent suspension of washed guinea pig corpuscles into foetuses of the same age, and 3. tests to determine the passage through the placenta of materials from mother to foetus.

An injection of 2 cc. of titer 500 hemolysin into the foetuses gave no reaction when the blood of the mother was tested for this hemolysin. The same quantity of material injected into a nonpregnant individual gave positive reactions over a considerable period of time. The hemolysin could be recovered from the embryos, for forty-eight hours, after which the tests were negative. The experiments were controlled by testing the maternal blood before the injection.

When 50 per cent suspension of washed guinea pig corpuscles was injected into rat foetuses, there was a response, on the part of the foetus as shown by tests for hemolysins and agglutinins in the foetal blood. The maternal blood showed, however, no increase in hemolysin either in the general circulation, or in the uterine vein blood. Instead, a substance was found which retarded and prevented the hemolytic reaction. This substance measured 25 units in the uterine vein, and as high as 1 to 2 units in the general circulation. The unit of measurement consisted of the prevention of the hemolytic process of 0.01 cc. of hemolysin (titer 500) by 0.1 cc. of serum.

When females were injected to produce hemolysins before conception, the hemolysin was not passed to a subsequent litter in demonstrable quantity, although the titer of the maternal serum was as high as 300 or 400.

Natural hemolysins, when found in the maternal serum, could not be demonstrated in the young.

The placenta of the white rat acts as a barrier and not as a semipermeable membrane to hemolysins prepared in the rat against guinea pig corpuscles. In some way the characteristic reaction of the hemolytic body is changed or else an antibody or neutralizing substance is formed, probably by the placenta. There is, of course, a possibility of this substance being formed in both the mother and the foetus but if such is the case, it is probably in much smaller quantity than that which is formed by the placenta.

The placenta of the white rat, therefore, restricts the passage either from mother to foetus or foetus to mother of natural hemolysin and of both actively and passively acquired white rat-guinea pig hemolysin.

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3488

Hormone Content of the Placenta and Chorionic Membranes.

EDGAR ALLEN.

From the Department of Anatomy, University of Missouri.

Earlier investigators have studied the hormone content of the placenta by the reactions of the mammary glands[1] and uterus[2, 3, 4] to injected extracts. It has been difficult to use such test reactions quantitatively. In collaboration with Dr. Doisy and others the writer[5, 6, 7] has shown that lipoid extracts of placenta will induce full oestrous growth in the vagina of the spayed rat and mouse, as will certain ovarian extracts. This reaction offers the following distinct advantages: (1) it affords several observations upon the living animal at definite intervals instead of a single one at autopsy for the determination of results; (2) it substitutes a maximum reaction which may be obtained in a shorter time interval (two days) for the partial reaction used in some of the earlier work; and (3) its clear cut end-point makes possible the quantitative utilization of this test for the biologic standardization of extracts in rat or mouse units.

Most of the extracts of placenta used in earlier work have been made from human material. Recently it was demonstrated that extracts of human placenta were as effective as follicular hormone from pig ovaries in inducing experimental menstruation in ovariectomized monkeys.[8] Why the placenta should contain a substance similar to the essential hormone of the ovaries and to what extent these endocrine functions are related are fascinating problems. In the case of the opossum, from the nature of the embryonic membranes, such a hormone is probably not necessary to the mechanism of the pregnant cycle. The great diversity among mammals of structural modifications of the tissues making contact between mother and embryo would lead one to look for wide variation in this endocrine function. It is reasonable to suppose that some of the mammals might reproduce without it. Therefore the range of variation of hormone content of the placenta, or the chorionic membranes in animals where no definite placenta is formed, should be determined.

Working toward a solution of this problem, quantitative tests of the hormone content of various tissues of pregnancy from different species of mammals are being made as material becomes available. To date, extracts of placentas or chorionic membranes from the cow, sheep, horse, pig, dog, cat, rat and chick have been tested. Eventually enough data may accumulate to at least outline the range of hormone content of these tissues.

The human placenta at term, weighing from 460 to 700 or more grams may contain as much as 200 to 400 or even more rat units of active substance.[6] A few analyses of placentas earlier in gestation have been made, and others are being added as specimens are received.[9] Extracts of two human chorionic vesicles obtained from ectopic pregnancies of less than two months' standing, have given positive tests in mice. These tests are interesting as the material extracted represents that derived entirely from the fertilized ovum. Positive tests have resulted also from extracts of material curetted from the uterus which showed placental structures on histologic examination.

The cotyledonary placentas of the cow* have shown the presence of this hormone in considerable quantity. The full term 'after-birth', representing of course only the embryonic part, has returned positive tests. Earlier in the gestation period it is possible to separate the maternal and embryonic parts of the cotyledons. This separation has been made on the killing floor of the packing house as soon as possible after the animals had been killed. While the embryonic part may be completely freed from maternal tissue, it is probable that tips of the chorionic villi may have been retained in the maternal pits. Both parts have returned analyses of nearly equal amounts of active substance.

Similar tests are at present being made of cotyledonary placentas from sheep.

Tests have also been made of full term chorions from the horse. This type of chorion with its diffuse arrangement of villi (representing of course only embryonic tissue) contains considerable amounts of active material.

Chorions of pigs taken during the latter half of the gestation period have so far returned uniformly negative tests. Extracts of the mucosa stripped from the uteri have been negative. It is worthy of note that in the pig the maternal embryonic relations are less intimate than those of the other mammals so far tested.

Analyses have been made of the zonular placentas of dogs and cats during the latter half of gestation. So far all tests have been negative. In some of these experiments the 'green borders' have been clipped off from the placentas before extraction because of the high solubility of their contents in lipoid solvents. Additional tests are desirable before a definite statement of the absence of hormone from zonular placentas is made.

In a single experiment an extract of rat placentas from five litters removed late in gestation failed to give positive tests in mice.

In a preliminary series of tests of small amounts of tissue from chicks[10] at different stages of incubation results have been negative.

It is highly desirable that additional tests of placentas and chorionic membranes from various animals at different stages of gestation be made to complete a survey of the distribution and range of this material. Such studies should provide sound evidence for interpretation of the relation between the endocrine functions of ovaries and placenta in the higher mammals. The publication of quantitative data will be deferred until the completion of additional tests.

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* I am indebted to Professor Andrew Uren of the Department of Veterinary Science, University of Missouri, for full term material from cows, horses, and sheep.

This is a preliminary report.

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3489

Degeneration in Vitro of Leucocytes and Connective Tissue Cells Under the Influence of Light.*

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From the Department of Anatomy, Vanderbilt University Medical School.

In an attempt to keep tissue cultures of certain blood cells under constant observation, work was completely blocked by the exceedingly rapid and extreme degeneration of the cells. In this rapid degeneration all of the various types of leucocytes showed degenerative changes rather similar in character, which differed, however, in certain particulars, for each individual type of cell. Of the changes shown by the various types of leucocytes, those shown by the polymorphonuclear neutrophil were the most striking. The neutrophils rapidly became very amoeboid, and at the same time their cytoplasm became more fluid, as was shown by the exaggerated brownian movement of the cytoplasmic granules. Soon cellular movement ceased; the cells rounded and became spherical. The polymorphic lobulated nuclei became edematous, rounded, but after a short while began to shrink and became reduced in size, although they still maintained their spherical or slightly ellipsoidal shape. The cells became swollen and edematous, and increased tremendously in size; in several instances the diameter of the cell reached three times that of the cell when it had first become rounded. During the development of this edema a number of minute oil droplets, giving a positive Sudan III reaction, became visible in the cytoplasm. From this stage on, two terminations to the process have been observed: (1) the cytoplasm gradually coagulated around the nucleus, leaving the cell membrane as a shell which slowly autolyzed; (2) the edema became so great as to rupture the cell membrane. At the moment of rupture the dancing granules and oil droplets of the cytoplasm almost instantly coagulated, evidently from contact with the surrounding medium.

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* This work has been assisted by a grant from the Henry Strong Denison Medical Foundation.

Without here going into details of the degenerative changes of the other types of leucocytes, it may be stated that they too followed a more or less similar, though less spectacular course of hydropsical degeneration.

This rapid degeneration has been noted in cultures of leucocytes from guinea pigs, cats and rabbits. In the preparation of the cultures the blood has been drawn under light ether anesthesia, either by heart puncture or through the abdominal aorta. It has then been centrifuged at low speed at room temperature in order to separate the leucocytes, which appear as the characteristic buffy coat. It is from this buffy coat that the hanging drop cultures have been made. Various culture media have been used including Tyrode solution, autogenous serum, autogenous plasma and autogenous heparin plasma plus chick embryo juice in Tyrode solution. The hydrogen ion concentration of all of these media has been set to approximately pH 7.4. It should be noted that in all of these media some red blood cells were brought over with the explant at the time of inoculation.

After the elimination of various preliminary factors which could have been responsible for such a rapid degeneration of the cultures as that described above, including the vaseline used to seal the cultures, cedar oil used for immersion objectives, the temperature at which the cultures were kept and the handling to which they were subjected, it was determined that in almost every case of such degeneration there was a definite record of exposure of the culture, for at least a minute or so, to some fairly concentrated light source, as for instance, a microscope lamp.

With this as a point of attack, an examination of over 700 cultures of white blood cells has shown that exposure of the cultures to rays from a light source (various types of incandescent electric globes) of such intensity as would be used in ordinary visual microscopic work, with the oil immersion lens, results in the rapid and extreme degeneration of the cells. To show how extreme and rapid this degeneration is, the increased motility of the leucocytes, due to the action of light, has been noted a number of times in cells after less than 10 seconds exposure to a light source such as that noted above. In other cases, an exposure of the leucocytes to such a light source, during a preliminary microscopic examination of 1 or 2 minutes, has resulted in the culture showing few or no living cells when examined a number of hours later. In all of these instances, controls from the same set of slides, kept in the dark, have remained normal.

Further, using an optical system which blocked out practically all of the ultra violet and infra red rays, it was determined that this degeneration under the influence of light was caused by the visual part of the spectrum. Using a constant light source, together with red (No. 29), blue (No. 45) and green (No. 58) Wratten light filters, it was further determined that the leucocytes underwent the same type of degeneration upon exposure to light of each of these three parts of the spectrum.

As stated above, all of these cultures contained at least some erythrocytes. This being true, there was a possibility that the leucocytes showed degenerative changes as a result of toxic substances produced by the action of light on these red blood cells. In order to analyze this possibility, cultures of leucocytes were inoculated with autogenous red blood cells which had been previously exposed to the action of intense light for about 5 hours. These cultures were then kept in the dark until examined. Upon examination at the end of 12 hours, the cultures showed no signs of any degeneration, such as that produced by the action of light upon white blood cells, indicating that the degeneration of the leucocytes as described above was not produced by toxic substances resulting from the action of light on the erythrocytes.

It next seemed desirable to determine whether this degeneration produced by the action of light upon the leucocyte was an intrinsic property of the cell itself, or whether it was the result of sensitization of the cell by some substance, *e. g.*, hemoglobin, furnished by the accompanying red blood cells. Inasmuch as at that time we were unable to obtain leucocytes free from red blood cells, recourse was had to fibroblasts cultured from chick embryos of from 4 to 14 days incubation. These fibroblasts from the heart, trunk and intestine of the chick embryos, grown in Tyrode solution, were exposed to the same light source which caused death of blood cells in from 5 to 80 minutes. At the end of 18 hours the fibroblasts were practically normal. On the other hand, cultures from the same groups of slides, inoculated in Tyrode solution plus autogenous washed red blood cells, when exposed to the same light source, showed a striking degeneration in from 1 to 3 hours. In this process of degeneration the cell either became packed with water vacuoles, or else the cell contents showed the same increased fluidity as that shown by the degenerating leucocytes. In both cases the cells gradually rounded, and both nucleus and cytoplasm became edematous. As may be seen from these observations, this type of degeneration of the fibroblast appears to be practically identical in nature with that described for the leucocyte under the influence of light.

Furthermore, it was found that these sensitized fibroblasts reacted to the red, the green and the blue components of the visible spectrum, and to the white light itself (the infra red and the ultra violet being blocked out), in a manner wholly comparable to that shown by the leucocytes.

This is a preliminary report.

3490

On the Experimental Production of Showers of "Non-Motile" Leucocytes.*

L. A. BEARD and J. W. BEARD. (Introduced by R. S. Cunningham.)

From the Department of Anatomy, Vanderbilt University Medical School.

Schilling,[1] in 1908, while studying the degeneration of white blood cells under the dark field microscope, found that dying leucocytes showed a definite swelling of the nucleus, and a change in the size and refractivity of the specific granules. Similar changes were later described by Sabin[2] as taking place in the death of the polymorphonuclear neutrophilic leucocytes. In the latter case, these changes were observed while studying the white blood cells by means of the supra-vital technique. Sabin was able to follow every step in the change from the living, motile polymorphonuclear neutrophilic

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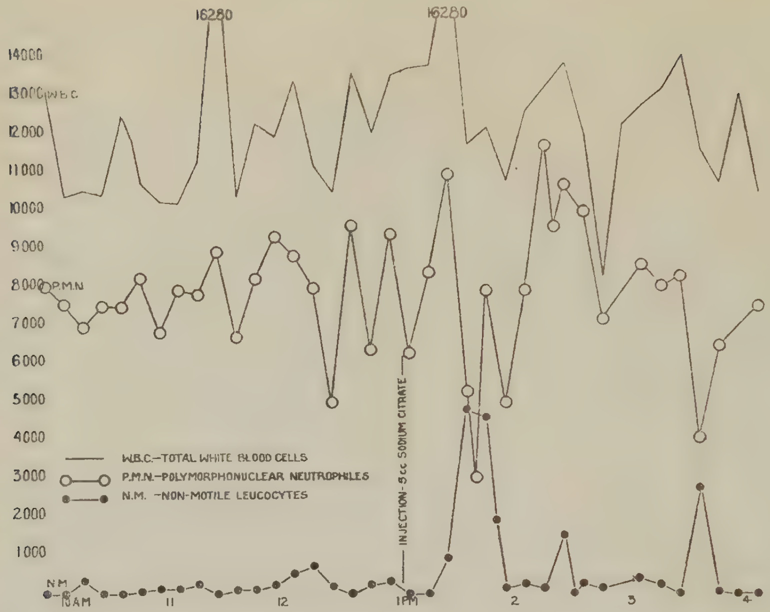
* This work has been assisted by a grant from the Henry Strong Denison Medical Foundation.

leucocytes to the dead, unstained cells, which she called "non-motile" leucocytes. She found that these changes could be produced by such technical means as pressure and heat, but after elimination of these errors she still found characteristic non-motile degenerating forms, and concluded that they represent a normal stage in the death and disintegration of the leucocytes. At the same time, she observed that these non-motile cells tended to appear in small showers, rather than in constant numbers. Later, in a detailed study of the normal rhythm of the white blood cells in the human, Sabin, Cunningham, Doan and Kindwall[3] confirmed the above observations concerning the appearance of non-motile cells in showers. They found in addition that these showers occurred at approximately hourly intervals.

In the course of a series of experiments in which we were studying the qualitative effect of sodium citrate, given intravenously, on the white blood cells, we were impressed by the large numbers of non-motile cells which regularly appeared following the administration of the citrate. A series of detailed experiments was then carried out to determine the exact character of these showers. It was found that the intravenous injection of a moderate amount of sodium citrate was uniformly followed by non-motile showers, which were many times greater than those seen in the normal rabbit. These showers regularly maintained the hourly rhythm, and were usually preceded by a definite clumping of the neutrophilic leucocytes. In several experiments the platelets were also counted, and were found to rise temporarily with some indication of a correlation with the showers of non-motiles. The supra-vital technique was used throughout the experiments for the differential counts.

The chart shows the results obtained in one of these experiments. The preliminary counts were made at 10 minute intervals over a period of about 3 hours, and during this time there were 3 small showers of 3 per cent, 2 per cent and 7 per cent respectively. During the 3 hours following the injection of 5 cc. of a 1 per cent solution of sodium citrate, 3 showers were obtained of 41 per cent, 18 per cent and 23 per cent; the striking finding being the maintenance of the normal hourly rhythm, with the great increase in the size of the individual showers.

In experiments in which comparatively larger doses were given, the normal rhythm was varied by the extension of the individual showers, so that there was a marked shortening of the intervals between them. Thus, some of the more extreme effects gave a marked instability of the curve, with non-motile cells present in



varying numbers for several hours following the injection. The amplitude of these showers of non-motile cells was uniformly far beyond that of any showers which we have observed in normal rabbits or of those reported by Sabin *et al.*[3]

By no technical or mechanical means has it been possible to induce such large numbers of degenerating leucocytes without producing general effects on other cells. This fact, taken together with the observation of the clumping of leucocytes in preparations preceding the shower of non-motile cells, with the admixture of normal active cells and non-motile cells in every preparation, and with the marked regularity with which these showers followed the injection of citrate, makes the conclusion seem unavoidable that these forms are produced within the circulation.

Any explanation of this phenomenon which may be advanced at the present time must be considered as only an hypothesis. It is possible that this great increase in non-motiles is the result of the injury to normal leucocytes, occurs either by direct action of the citrate on the cells, or indirectly through the action of the citrate on some mechanism capable of causing this effect in the organism. On the other hand, it is also possible that there is some storehouse where they may normally be formed or collected, from which they are expelled into the circulating blood by the action of sodium citrate on some mechanism, operating normally to produce the usual small showers of these degenerated leucocytes which are found in the peripheral blood stream. Experiments are at present in progress to determine the exact nature of this mechanism.

This is a preliminary report.

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3491

The Relation of Calcium to the Toxicity of Carbon Tetrachloride in Dogs.*

A. S. MINOT. (Introduced by P. D. Lamson.)

From the Department of Pharmacology, Vanderbilt University Medical School.

In the treatment of several million cases of hookworm disease with carbon tetrachloride a few deaths have occurred. Some of these were probably due to the taking of alcohol with the drug, and others to mechanical obstruction by ascaris worms. In a very few instances, however, carbon tetrachloride was apparently the direct cause of death. In an attempt to explain these cases extensive studies have been carried out by Lamson and his associates.[1] These authors found that it was practically impossible to produce visible signs of intoxication in dogs by the oral administration of carbon tetrachloride in doses even up to the capacity of the stomach (250 cc.). Despite the general normal condition, pathological examinations and various functional tests showed that even single small doses caused considerable damage to the liver, while typical cirrhosis of the liver could be produced by repeated administration.[2, 3, 4, 5]

The work was transferred in 1925 to the Vanderbilt University Medical School. Continued studies here with various liver function tests gave results quite analogous to those reported in the earlier papers. The death of a great number of experimental animals, however, indicated that some new factor had been introduced in spite of the fact that the same carbon tetrachloride was used. In contrast to the earlier results, doses as low as 4 cc. per kilo body weight (the smallest amount capable of influencing the phenoltetrachlorpthalein test) now proved fatal in practically all cases, while attempts to produce cirrhosis with repeated doses were futile because the subjects all died.

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* This investigation is one of a series of studies being made under the direction of Dr. P. D. Lamson on the pharmacology and toxicology of carbon tetrachloride. The work is being carried on with the support of the International Health Board.

The usual symptoms in the dogs in this laboratory, after receiving 4 cc. of carbon tetrachloride per kilo by mouth, were a loss of appetite and a noticeable lack of energy during the first 20 to 30 hours. Some of the dogs then rapidly became unconscious, or so sluggish that they could be aroused only with great difficulty. Others, and these were more common, showed muscle twitchings, starting as a fine tremor of the toes and lips and increasing in severity over a period of several hours, until nearly the entire musculature was involved in typical tetany. Frequently the diaphragm was also involved, causing spasmodic breathing. Death occurred in either type 40 to 60 hours after the drug was administered.

The similarity of the convulsive symptoms to infantile tetany, as well as the fact of their appearance only after the work was transferred to Nashville, suggested a possible calcium deficiency in the new diet. During the previous experiments the food had been a well balanced ration consisting of meat, corn-meal, and bone meal baked into a cake. Here the diet had been almost exclusively lean beef scraps containing very little bone. There had been, therefore, a very radical decrease in the calcium intake. Nevertheless dogs which did not receive carbon tetrachloride seemed to be well nourished on this new diet. Apparently some condition caused by the carbon tetrachloride was making unusual demands on the organism which could not be met by animals kept for some time on a diet deficient in calcium.

The most consistent change found in dogs after the oral administration of carbon tetrachloride was a marked increase in the concentration of bilirubin in the blood as shown by determination of the icteric index.[6] This constituent, which is normally present only in traces in dog's blood, began to increase about 12 hours after the dose was given, and the more severe signs of intoxication appeared soon after the bilirubinemia reached a high level. Several observations serve to link this change with the possibility of calcium lack. It has been shown that the slowed clotting time of blood in obstructive jaundice can usually be restored to normal by the addition of ionized calcium.[7] Furthermore, Bowler has reported that the lethal intravenous dose of calcium chloride is much higher for jaundiced than for normal dogs.[8] Earlier papers by King and Stewart[9] and King, Bigelow and Pearce[10] show that bile pigments combine with calcium in the blood. This protects the organism against the toxicity of bile pigments, but at the same time depletes reserve of calcium in the tissues and causes an increased elimination. There may be an actual increase in the total calcium present in the blood, but much of it is combined with bilirubin so that a decreased concentration of *ionized* calcium results. Conditions of tetany were not reported in any of these experimental studies. It seems quite possible, however, that while animals on an adequate ration may have a sufficient reserve of calcium to prevent such acute symptoms, nevertheless a removal of ionized calcium might readily be disastrous to animals kept for some time on a deficient diet.

To test this point calcium chloride was administered intravenously to dogs in the convulsive or unconscious stages of intoxication from carbon tetrachloride. Within 15 to 30 minutes the animals walked around the laboratory in a fairly normal condition. The muscle twitching disappeared temporarily. Relapses into the comatose or convulsive condition occurred frequently, but the response to intravenous calcium injections was always prompt. By repeated medication at intervals of 3 or 4 hours the animals could be brought through the crisis of the intoxication and completely cured. Relapses needing repeated medication recurred as long as the icteric index remained high, usually for 2 or 3 days. Furthermore, the simple addition of calcium salts to the meat diet for a week or more preceding the administration of carbon tetrachloride made the dogs here show the same tolerance for the drug as reported by Lamson.[1] Large doses, 4 to 6 cc. per kilo, which had been consistently fatal, now produced no sign of intoxication, except the usual liver damage as evidenced by liver function tests.

The oral administration of calcium salts has failed to save animals already showing signs of carbon tetrachloride intoxication. Death was delayed somewhat but the absorption of calcium was too slow to be adequate. The oral administration of ammonium chloride as an indirect means of influencing the blood calcium[11, 12] was more effective. When the drug was retained the animals recovered. Persistent vomiting in some cases, however, made it necessary to resort to intravenous calcium therapy.

These results show that (1) carbon tetrachloride is relatively very toxic for dogs that have been on a prolonged low calcium diet; (2) signs of intoxication can be prevented by the continued addition of calcium to the diet; or (3) cured either by the intravenous injection of calcium chloride, or the oral administration of ammonium chloride.

These observations all suggest that the immediate cause of death in these dogs may be attributed to the lack of ionized calcium in the blood, secondary to the bilirubinemia, following the administration of carbon tetrachloride. Furthermore, the possibility that the production of serious symptoms by the lack of dietary calcium might occur as readily in any other jaundiced condition, may be a point of considerable clinical importance.

Further experimental work is being done regarding the total and ionized calcium in the blood following carbon tetrachloride administration and in other jaundiced conditions, and also on the effect of the parathyroid hormone as a therapeutic agent. This work together with the experimental data already accumulated will follow this preliminary note in a later publication.

In man uncomplicated deaths from carbon tetrachloride have been so rare that there is no typical picture of intoxication. It is, however, known that such deaths occur 24 hours or more after the administration of the drug. Convulsions have been reported in some cases of poisoning. The majority of these cases have occurred in children in whom calcium deficiency is a common clinical condition. Whether deaths following anthelmintic treatment can be attributed to a calcium deficiency remains to be investigated, but the foregoing experiments would indicate the advisability of an adequate calcium diet for a considerable period preceding carbon tetrachloride administration, and suggest the possibility of calcium therapy in cases of acute intoxication.

This is a preliminary report.

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3492

Note on the Pharmacology of Ergothioneine.

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A new sulfur-containing compound has been recently isolated from the blood[1] which was identified[2] as ergothioneine, first obtained by Tanret[3] from ergot, and later shown to be the betaine of thiolhistidine by Barger and Edwins.[4] The only information we have found in the literature as to its pharmacologic action is a single sentence in the paper of Barger and Edwins[4] to the effect that the product is pharmacologically inactive. Since there is no statement as to the functions tested, it seemed advisable to reinvestigate this compound pharmacologically. We are indebted to Professor T. B. Johnson for our supply of the material.

Doses of 0.1 to 0.2 gm. per kilo injected subcutaneously in rabbits produced no change in the level of blood sugar over a period of 4 hours, as determined by the Hagedorn-Jensen method. In addition, there was no observable increase in respiration, pulse rate, nor diameter of the pupil following these rather large doses. In the cat, under amytal anesthesia, amounts ranging from 1 to 60 mgm. per kilo, intravenously, produced no change in the blood pressure, pulse rate, respiration nor biliary secretion. Excised strips of the cat's duodenum showed no alteration in tonus, rate, nor amplitude with concentrations in the bath up to 0.06 per cent ergothioneine.

Whatever may be the metabolic significance of ergothioneine, the negative results of the above experiments support the earlier conclusion that the substance has no well-marked pharmacological actions.

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* Fellow of the National Research Council in Medicine.

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3493

Automaticity and Mechanical Work in Anoxybiontic State of Frog's Heart; Quantitative Estimation of Natural Excitations.

LEON ASHER.

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A quantitative method was worked out for the registration of the stroke volume of the frog's heart under as nearly normal physiological conditions as possible, with the sole exception that oxygen was rigorously excluded. Carefully purified hydrogen was perfused continuously through the circulatory system, and through the bath which contained the heart, from which the stroke volume was recorded by the Asher recorder. As the pressure was observed in an arterial manometer, the work of the heart could be computed. The system contained venous and arterial valves. The pressure on the venous inflow-side, and the arterial resistance could be varied. The perfusing fluid introduced by Brömser, Barkan and Hahn was used.

In this system automatic beat could be observed for 61 hours, notwithstanding that for long periods the heart had been forced to beat more rapidly and do more work than normally. These observations demonstrate conclusively the possibility of the persistence of automatic excitations without oxygen for a much longer time than is stated in the current literature. If one could exclude the necrobiosis of the small fragments of tissue tied to the canula, the automatic beat would probably last longer.

The principal conditions for the persistence of a good automatic beat are the right composition of the circulating fluid, a periodical renewal of the bath fluid, low pressure on the venous side, an optimal arterial pressure, a constant perfusion of hydrogen, and especially the maintenance of circulation in case the beats begin to fail. Artificial beats of the heart, by a pump arrangement, restore the heart invariably.

The amount of work done by the heart and especially the question of fatigue and restoration was then examined. For over two days the anoxybiontic heart could maintain a fairly normal circulation. Perhaps of still greater importance is the fact that even restoration was possible, notwithstanding the lack of oxygen. By forcing the heart through artificial stimulation to a more rapid rate of beat over a number of hours there appears fatigue. This, from numerous investigations on skeleton-muscles in the Berne physiological institution, we have called *apparent fatigue*. For as soon as the rate of beat is diminished through change to a less frequent rate, or through letting the automatic excitations act on the heart, the beats are augmented, and in a short time become the same as before. As our method permitted measurement of the work done, it was found that during the state of apparent fatigue less work was done and the minute volume was diminished. Re-establishing a normal rate also raised the work done and the stroke volume to return to normal. The changes from one state to the other could be repeated many times during a period of over 48 hours. The only difference between restoration in absence or presence of oxygen is that in the latter case restoration is still more rapid.

The possibility of restoration of the frog's heart without oxygen marks clearly a deep difference between the biochemical mechanism of the heart and skeleton muscle. The researches of Hill and Meyerhof have taught the importance of oxygen in the phase of restitution for the skeleton muscle. Various researches in the Berne physiological institution have shown that under physiological conditions the skeleton muscle is practically not fatiguable, *viz.*, can be restored from an apparent fatigue through the sole change from a more frequent to a less frequent contraction. All these studies were made on the intact animal. But even for the isolated sartorius muscle, we were able to create physiological conditions, so that after many hours of incessant work the muscle did not become inefficient. But in all these experiments oxygen was necessary. If the bath of the muscle was perfused with hydrogen a slower rate of contractions was not able to restore the muscle.

Incidentally, on studying the work of the heart under lack of oxygen, observations were made on a fundamental problem of normal excitations. As already mentioned, frequently artificial stimulations were applied, by aid of the calibrated Kronecker inductorium. The longer the heart had to work under exclusion of oxygen, the more the excitability towards artificial stimulations was lowered. Sometimes even 13,000 units of the Kronecker inductorium failed to stimulate. But on interrupting the artificial stimulations automatic beats set in, thereby showing that the natural excitations are more potent than the artificial ones. We have here gained, perhaps for the first time, a quantitative method to estimate the equivalent intensity of natural excitations. I prefer to use the term equivalent intensity to avoid for the moment the hypothesis that the potential difference of natural stimuli are the same as those of the alternating current of the Kronecker inductorium. The greater potency of natural stimuli has been observed already in the investigations of Sherrington and Graham Brown, when artificial stimuli were unable to call forth a reflex movement, but the natural stimuli arising in the spinal cord produced movements. In the case of our heart experiments the possibility appears of a fairly quantitative measurement.

3494

On the Ionization of Calcium Citrate.

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The hypothesis that citrate ions combine with calcium ions in some way and thus remove them from solution serves to explain a number of observations. Among these may be mentioned the following: non-precipitation of calcium phosphate in alkaline solutions when magnesium citrate is present; the failure of Shipley, Kramer and Howland[1] to obtain calcification *in vitro* even with a Ca x P product of 60 when the calcium is present as calcium citrate; the unpublished observations of Shelling and Maslow in this laboratory on the production, by intravenous injection of sodium citrate, of convulsions clinically similar to those of tetany, but with a normal total serum calcium and a greatly increased dialyzable calcium; and the results reported very recently in the important contributions of Hastings, Murray and Sendroy[2] on the solubility of calcium carbonate and calcium phosphate.

There are no studies, however, which bear directly on the ionization of calcium citrate. We are, therefore, reporting some experiments which give direct evidence in favor of the above hypothesis.

Conductivity titrations were performed using N/200 calcium chloride and N/200 sodium citrate solutions. The conductivity of each solution was measured, that of the citrate being found the greater. Measured volumes of the citrate solution were added to a measured volume of the calcium solution. The conductivity of the resulting mixture was measured after each addition. The first addition of the citrate produced a decrease in conductivity instead of an increase. Further additions of citrate caused further decreases. It was only after an equivalent quantity of citrate had been added that subsequent additions caused the conductivity to increase. The decrease in conductivity was marked; when an equivalent quantity of citrate was added, the resistance of the mixture was about 50 per cent higher than the value which would have been obtained had there been no ionic reaction.

Such decreases in conductivity are attributed to a decrease in the number of ions in the solution. To rule out any decrease in the number of hydrogen ions or hydroxyl ions, the pH of both solutions was first adjusted to 7.4 with N/10 sodium hydroxide or N/10 hydrochloric acid. The amount of sodium chloride formed in each case was quite small. The behavior of sodium chloride was, nevertheless, studied and was found to be normal, as was expected. Conductivity titration of N/800 sodium chloride with N/200 sodium acetate gave values which decreased steadily from that of the sodium chloride solution toward that of the acetate, as calculated on the assumption that no ionic reaction occurred.

The marked decrease in conductivity observed when a solution of sodium citrate is added to a solution of calcium chloride is, therefore, interpreted as being due to the removal from the solution of the calcium ions by the citrate ions. Un-ionized molecules of calcium citrate are probably formed although the formation of a complex ion of some sort is another possibility to be considered.

This is a preliminary report.

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SCIENTIFIC PROCEEDINGS.

New York Meeting.

College of the City of New York, April 20, 1927.

3495

Secondary or Compensatory Hypoglycemia.

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In contrast with true hypoglycemia, of which insulin hypoglycemia is an example, we suggest the term secondary or compensatory hypoglycemia for those conditions in which an initial rise in blood sugar is followed by a fall below normal. McGuigan and Ross[1] state that many investigators have reported a hyperglycemia, while others working with the same substance have demonstrated a hypoglycemia. Santos[2] finds that secretin exerts a hypoglycemic action in man, dog and rabbit. Lambert and Hermann,[3] however, show that injection of secretin causes transient hyperglycemia, followed by hypoglycemia. Such differences in observation may be due to the quantity of reserve glycogen in the animal, or to the time of withdrawal of the blood for analysis, after injection of the biologic mixture or compound under investigation.

We postulate that any biologic condition or chemical substance giving rise to persistent hyperglycemia may eventually produce hypoglycemia. Josephs[4] has demonstrated that the hyperglycemia developed during anesthesia results in post-anesthetic hypoglycemia. According to Herold,[5] a high blood sugar during obstetrical labor is followed by post-partum hypoglycemia. Maud L. Menten[6] has reported that the soluble toxic products of the enteritidis-paratyphoid B group produce an initial hyperglycemia and a secondary hypoglycemia. Taya[7] has shown that a rise in temperature causes an increase in blood sugar concentration, followed by a fall below normal. Geiger[8] maintains that spontaneous fall of fever is accompanied by a hypoglycemia.

Adrenalin, known to cause a marked rise in blood sugar, is also capable of producing hypoglycemia. According to György and Herzberg,[9] the initial hyperglycemia occasioned by adrenalin is finally superseded by hypoglycemia. The latter results from depletion of the glycogen stores incident upon the increased glycogenolysis and increased glucose consumption. Collens, Shelling and Byron[10] found that ligating the hepatic artery causes a total depletion of the glycogen reserves of the body. During this process adrenalin injected intravenously has less and less effect the longer after ligation it is injected. When the hypoglycemia has developed, adrenalin does not affect the sugar level of the blood. According to Gatin-Gruzewska,[11] the liver and muscles in the rabbit can be rendered glycogen-free by injecting adrenalin after a one-day period of starvation.

The hormone of the thyroid gland behaves like the adrenal hormone with reference to its influence on blood sugar. Marks[12] followed the blood sugar curve of rabbits given thyroid extract. After the injection of glucose (1/6 gm. per kilo), there first appeared, in comparison with the controls, a definite rise in blood sugar during the first and second hours. The initial rise was followed, however, by a marked drop in blood sugar. If during the hypoglycemic stage no additional sugar was given, the animals became convulsive, went into coma, and finally died. Schwarz[13] recently reported a case of pregnancy complicated by hyperthyroidism, in which hypoglycemia developed.

Rabbits and frogs can also, according to Kulz, Zuntz and Vogelius, Frentzel[14] and others, be rendered glycogen-free by injections of strychnine. This alkaloid is known to cause hyperglycemia. If it can desugarize animals it should be able to induce secondary hypoglycemia. The following experiment proves the correctness of this supposition:

Morphine Sulphate.

Weight of rabbit, 1370 grams.

Dose: 30 milligrams per kilo, injected subcutaneously.

	Blood Sugar.
Before injection	0.124%
1½ hours after injection	0.167%
2½ hours after injection	0.104%
3½ hours after injection	0.060%
4½ hours after injection	0.060%

Since morphine produces hyperglycemia, it should also give rise to secondary hypoglycemia. The following experiment bears out the *a priori* reasoning:

Strychnine Sulphate.

Weight of rabbit, 2000 grams.

Dose: 3 milligrams per kilo, injected subcutaneously.

	Blood Sugar.
Before injection	0.104%
1 hour after injection	0.150%
3 hours after injection	0.118%
5 hours after injection	0.066%

Hydroxylamine hydrochloride we have found to behave like morphine and strychnine. As the experiment below shows, it gives rise to hyperglycemia and secondary or compensatory hypoglycemia.

Hydroxylamine Hydrochloride.

Weight of rabbit, 2150 grams.

Dose: 12 milligrams per kilo, injected subcutaneously.

	Blood Sugar.
Before injection	0.092%
1 hour after injection	0.138%
3 hours after injection	0.118%
4 hours after injection	0.079%
5 hours after injection	0.060%

When two substances giving rise to hyperglycemia are administered, the result is a summation effect, and secondary hypoglycemia may be more readily produced. Diesel[15] reports that extracts of pituitary reduce the hyperglycemia developed by adrenalin. Ross[16] states that ether given after morphine does not increase the blood sugar as much as if ether alone had been administered. György and Herzberg[9] find that the preliminary administration of ammonium chloride increases the secondary hypoglycemia produced by adrenalin. Ammonium salts, as the experiment suggests, may have a tendency to produce hyperglycemia.

Ammonium Carbonate.

Weight of rabbit, 1600 grams.

Dose: 25 milligrams per kilo, injected subcutaneously.

	Blood Sugar.
Before injection	0.098%
45 minutes after injection	0.167%

Among other substances producing initial hyperglycemia and secondary hypoglycemia may be mentioned thallium compounds (Mamoli[17]), hydrazine (Underhill and Karelitz[18]), chloroform and phosphorus (Bodansky[19]). Very recently Izume and Lewis,[20] working with hydrazine and its derivatives, observed that when hyperglycemia and hypoglycemia were present the hyperglycemia occurred first, following a period of excitement, and the hypoglycemia later, during the stages of depression.

Hypoglycemia may be due to an increase in the rate of disappearance of the sugar in the blood resulting (1) from greater combustion of glucose (glycolysis), or from using up the glycogen reserves, as in fasting; (2) from greater withdrawal of glucose for storage purposes (glycogenesis); or (3) from greater elimination by the kidney of sugar (glycuresis) or its intermediate products of metabolism. Hypoglycemia may also be due to a decrease in the supply of sugar available in the system resulting (1) from interference with glycogenolysis (conversion of glycogen to glucose); or (2) from interference with glyconeogenesis (conversion into glucose of non-carbohydrate material, like amino acids).

Adrenalin causes secondary hypoglycemia because of glycogen depletion due to increased consumption of glucose. Lyman, Nicholls and McGann[21] state that this hormone causes a hyperglycemia accompanied by an increase in heat production and by a rise in the respiratory quotient. Hydrazine and its derivatives, according to Izume and Lewis, give rise to secondary hypoglycemia as a result of decrease in the supply of glucose available for combustion. At first hydrazine produces an increase in the consumption of carbohydrate. After the supply of reserve glycogen is used up, hypoglycemia results. No new sugar, or very little sugar, is formed as a result of the liver injury, which diminishes glyconeogenesis or the transformation of amino acids into glucose.

The mechanism of the production of primary hypoglycemia is probably not the same as that of secondary hypoglycemia. Substances producing secondary hypoglycemia should differ from insulin. In this connection it may be said that a review of the experimental data furnished by Collip[22] in relation to his plant glucokinin leads to the belief that it is not identical with insulin. Glycokinin gives at first a normal blood sugar or an increased blood sugar, and the hypoglycemia, which seems to be secondary, makes its appearance many hours or days after the injection. Insulin, on the other hand, yields an immediate hypoglycemia.

It is suggested that in all conditions where a secondary hypoglycemia is apt to occur, glucose or preferably glucose and insulin should be administered as a therapeutic measure. Such procedure should, therefore, be followed to prevent the symptoms of post-anesthetic or post-partum hypoglycemia, and in poisoning or overdoses of such drugs as morphine or strychnine.*

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* In all the experiments recorded the animals received no food except water for 24 hours prior to injection. Blood sugar was determined by the iodometric method of Shaffer and Hartmann.

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3496

The Influence of the Thyroid Gland on Experimental Rickets.

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From the Hull Laboratory of the University of Chicago.

Histological studies of sagittal sections through the distal end of the femur of rats 69 days old, and thyroidectomized between 15 and 29 days after birth, reveal a marked overgrowth of the epiphyseal cartilage close to the junction with the metaphysis. This area is also characterized by deficient calcification and invasion of blood vessels, as in typical rickets. Part of these rats were fed the Steenbock and Black[1] diet No. 2965. Cod liver oil added to this diet in amounts varying from 2 per cent of the diet up to a quantity where food became objectionable, did not prevent the development of rickets in these rats. Other thyroidectomized rats of the same age were fed a diet consisting of table scraps of the various kinds of food constituting the dietary of well-nourished individuals. These rats also developed rickets but in a somewhat lesser degree. Unthyroidectomized control rats of the same litter, living on identical dietaries, did not develop rickets, as determined by histological examination of the epiphysis of the long bones. These experiments demonstrate the necessity of the thyroid hormone for the complete utilization of cod liver oil.

This is a preliminary report.

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3497

Microbic Dissociation: The Tubercle Bacillus.

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The studies of dissociation of R and S colonies in various types of microorganisms suggested an investigation of a similar nature with the tubercle bacillus. Here we encounter many difficulties. Workers with tubercle bacilli are aware of the fact that it is almost impossible to prepare a suspension free of clumps and that there are very seldom individual organisms in the suspension. Secondly, as the organisms develop very slowly, sometimes requiring four weeks for a visible colony to appear, contaminations are liable to occur and spoil the investigations of even the most careful workers; and thirdly, it is almost impossible sometimes to prevent dehydration of the culture medium upon which the organism is cultivated. When the tubercle bacilli are cultivated on media prepared in Petri dishes contamination occurs in a large number of the plates. These contaminations are not due to the initial exposure during the seeding, but to the leakage of the air within the plates. In the incubator temperature the air is expanded and again contracted when the Petri dishes are removed to room temperature where the air rushes into the plates, carrying along the contaminating molds and other organisms.

In the following study we have used for subculturing the tubercle bacilli the author's gentian-violet-egg medium made up in Petri dishes. (The procedure for preparation of the medium and inspissation can be found in any text book.) The suspension of the organism was prepared as follows: Individual colonies were removed from the original culture and triturated with platinum wire on the inside wall of a small tube containing 2 cc. of sterile salt solution with a pH of 7.8. After trituration was completed, the tubes were rotated until all of the masses of the organism were dispersed in the salt solution. This was then filtered through two layers of sterile filter paper and the filtrate was inoculated on the surface of the medium mentioned above. To prevent evaporation and contamination we have adapted the following method which proved very satisfactory: Rubber bands 3½ inches long and 1½ inches wide were slipped over the edge of the Petri dish so that each half of the Petri dish was covered with ¾ inch of the rubber band, resulting in a good tight fitting.

Up to the present, two types of tubercle bacilli have been investigated. The B 1 strain is a tubercle bacillus of bovine origin of moderate virulence for rabbits, which have been in our possession

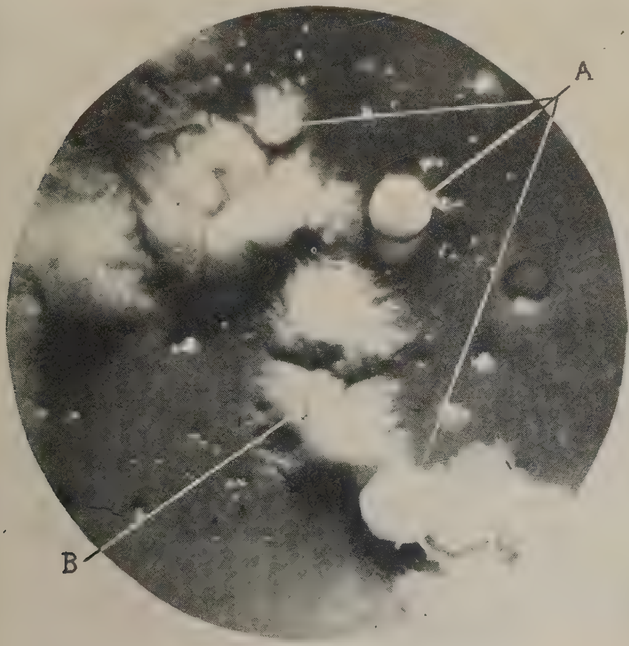

FIG. 1.

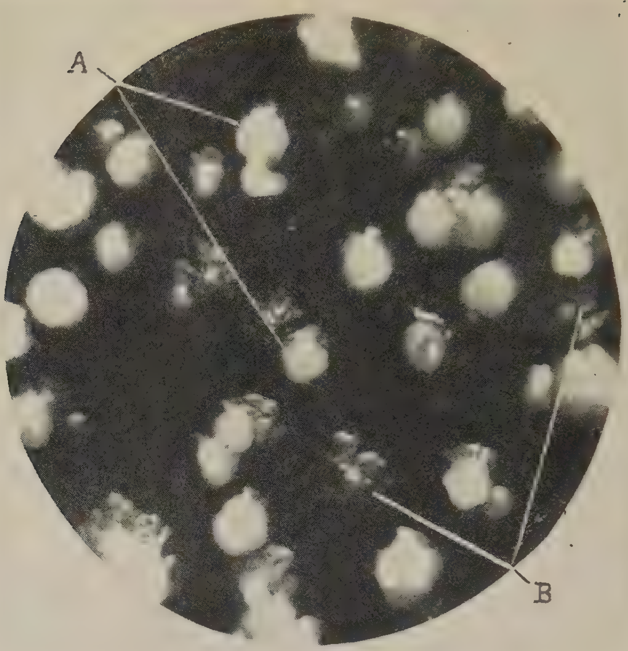

FIG. 2.

for a number of years. In the photograph it can be clearly seen that there are two distinct types or colonies, one (Fig. 1 A) being perfectly round, smooth, glistening and resembling small moth balls, while the second type of colony (Fig. 1 B) is very rough, irregular and dry, spreading right into the culture media. The second organism is of avian type, which was very kindly sent to me by Dr. Moore of Ithaca. Here, again, it is very evident from the picture that one (Fig. 2 B) of the colonies is almost transparent, rather irregular and spreading, while the other (Fig. 2 A) is more of the granular opaque type, very much raised and smooth. The former is moist, while the latter is dry.

At present work is in progress to study various phenomena which have been observed with R and S colonies with the other organisms. It seems that the tubercle bacillus in all probability does not vary in regard to "dissociation" from that seen in the other types of microorganisms.

This is a preliminary report.

3498

A Bacteriophage Active Against a Virulent Hemolytic Streptococcus.

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We reported[1] last year the finding of a bacteriophage against a virulent hemolytic streptococcus. Such a lytic principle active against streptococcus has been reported so rarely, if at all, that it seems desirable to state our results in some detail.

The organism against which the phage was originally active was isolated about 3 years ago from a spontaneous fatal infection in a stock rabbit. At autopsy, the animal showed marked thoracic lesions, myocarditis, pericarditis, bronchopneumonia, fibrino-purulent pleurisy, extensive empyema with relatively little grossly noticeable below the diaphragm save an acute splenic tumor. The organism was readily obtained in pure culture from these lesions and also from the heart's blood. It grows on sheep blood agar as a very moist, translucent, mucoid colony, somewhat similar to that of *Pneumococcus mucosus,* but it produces a wide zone of hemolysis on the plate. One cc. of a 12 hour broth culture will cause complete hemolysis of a 5 per cent suspension of washed sheep red blood cells when incubated for one hour at 37° C. It does not ferment inulin and is bile insoluble, so we consider it an example of a *Streptococcus mucosus.* It closely resembles the organisms found in cases of septic sore throat. The various fermentation reactions are recorded in Table I.

The organism is Gram positive, but is more readily decolorized than many streptococci, and, frequently, partially decolorized cocci may be observed in a chain of Gram positive organisms. It appears in chains of moderate length, the individual members of the chain usually being spherical and variable in size. Capsules can be readily demonstrated by any of the commonly used methods.

This organism proved virulent for other rabbits, causing a fatal septicemia in 5 to 7 days, when injected intravenously into full grown animals in amounts of .001 cc. of a broth culture. It has, over a period of three years, shown a strong tendency to localize in the thoracic organs, especially the heart, and has maintained its original virulence to a marked degree for many months without animal passage.

The common finding of the lytic principle in sewage led us to

TABLE I.

Fermentation Reactions.

	Dextrose	Mannitol	Maltose	Xylose	Lactose	Salicin	Sucrose	Raffinose	Dulcite	Inulin	Dextrin
Streptococcus mucosus (Rabbit) 48 hours	+	−	+	−	+	+	+	−	−	−	+
Streptococcus mucosus (Guinea pig) 48 hours	+	−	+	−	+	+	+	−	−	−	+

wonder whether the activated sludge process, as used in Milwaukee, Wis., with its unusually high content of aerobic spore formers, might not be a source of phage against other organisms than the common members of the colon (dysentery) typhosus group. This possibility seemed especially interesting because of the finding by Nicolle[2] that a filtrate of *B. subtilis* would dissolve pneumococci. Accordingly, a dozen different streptococci and pneumococci and half as many members of the colon typhosus group were grown in flasks containing 50 cc. beef infusion broth plus 5 cc. of the activated sludge. After 3 to 4 days' incubation this mixture was centrifuged, filtered through a Mandler filter, and tested for the presence of phage against the organism employed. If the result was negative, the filtrate was reinoculated with the organism and the activated sludge and the whole process repeated at least ten times. We very promptly obtained a lytic principle active against the different members of the colon typhosus group used, and on the second cultivation attempt we succeeded in obtaining the phage active against the *Streptococcus mucosus*. All efforts to obtain a lytic principle active against other streptococci and pneumococci failed.

This bacteriophage causes the transmissible lysis of this particular streptococcus, and in other ways acts in a typical manner. We have commonly employed standard beef infusion broth with a pH of 7.6 and the organisms from a broth culture from 5 to 24 hours old. The phage made up in increasing dilutions in this broth will inhibit the growth of the specific organism, *Streptococcus mucosus,* in dilutions usually as high as 1×10^{-9}, and occasionally as high as 1×10^{-13} when two drops of a turbid young broth culture are added to the phage dilutions and the whole incubated for 24 hours. Quantitative subculture platings on blood agar show that most of the tubes are not actually sterile, although the number of living organisms is small. We have obtained actual sterility only by using relatively large amounts of the phage, 10 per cent or 33⅓ per cent of the total volume of the broth. Not always have these concentrations given sterility. These concentrations have been more effective in giving complete sterilization than has 100 per cent phage.

On blood agar tubes or plates the phage causes a marked diminution in the number of colonies planted on a given area. Only rarely, however, does one observe the moth eaten type of colony so common with the phagic action on members of the colon typhosus group. This is due partly, we believe, to the small size of the colonies of this organism. When a drop or two of this phage is added to a tube of young broth culture of the organism, complete clearing of the tube takes place when incubated for a few hours.

We succeeded in obtaining a similar phage against this organism by the use of 2 other samples of the Milwaukee activated sludge, although once it took 6 cultivations to obtain this result, the filtrate from the fifth cultivation showing no phage action. We also succeeded in obtaining the phage upon the first cultivation with the effluent from Madison sewage, which is always a ready source of phage active against the various members of the colon typhoid group.

With the filtered products of its own metabolism, repeated cultivation of this streptococcus failed to produce any phagic action, even when recultivation was continued thirteen times.

Our notion that the aerobic spore formers of the activated sludge might be responsible in initiating the production of the lytic agent seems to be without foundation. We tried repeated serial cultivations of the streptococcus with a sterile filtrate of the proteases and digestion products from *B. subtilis* and failed to obtain any phagic action.

We endeavored to adapt our phage to 6 other hemolytic streptococci (2 of them isolated by G. Howard Brown from cases of septic sore throat) and to each of the 3 fixed types of pneumococci by all of the usual methods, and by various modifications of our own, but entirely without success.

The fact that our organism was obtained originally from a spontaneous infection in a rabbit, and is definitely virulent for that animal, has given us a favorable opportunity to try the effect of the phage in the treatment of a disease in its original host. A number of series of animals were, therefore, treated with the lytic principal. In 3 series, treatment was begun before the organisms were injected, and in 3 series, immediately after the injection of the organisms. Varying dosages of the phage were employed up to 20 cc. daily and various routes were used (*per os,* intravenous and intraperitoneal), but in no case did the treated animals do better than the untreated. In fact, in all instances in which large amounts of the phage were used, the treated animals died before the control animals, and showed the same type of lesions and a similar degree of severity.

We found no evidence of the passage of the phage into the blood stream when as much as 10 cc. was injected *per os* or intraperitoneally, nor did the phage remain in the blood stream any considerable length of time when injected directly into the vein. Tests of blood serum taken 2 hours and also 2 days after such treatments showed no bacteriophagic action. This elimination of the phage from the blood stream is due, at least partly, to adsorption of the phage directly by the blood cells. Experiments both with defibrinated sheep, and rabbit blood, plus the phage, incubated and shaken together for 2 hours, showed marked, sometimes complete, inhibition of the phage.

One of the obvious difficulties in any possible use of the phage as a therapeutic agent lies in the fact that the phagic broth must also contain various toxic elements from the metabolism and cleavage of the organisms. The separation, therefore, of the phage from these toxic products would seem to be a necessary step. We have tried both the acetone and the alcoholic precipitation methods described by Kabeshima,[3] but our phage was completely destroyed by the alcohol method, and markedly reduced in potency by the acetone method.

Various adsorption reagents were employed: ordinary kaolin;

Lloyd's reagent, and charcoals. The best adsorbing agent proved to be Rankanite A, an activated charcoal perfected by the Chemical Warfare Service during the war. (We obtained this through the courtesy of Professor Victor Lenher of the University of Wisconsin.) Twenty grams of this charcoal, combined with 100 cc. of the broth phage, shaken for one hour in a machine, and refrigerated over night, will completely adsorb the phage from the broth. Tests of the supernatant show no evidence of phage even when the broth had originally a titer of one to a billion.

After striving without success for the better part of a year to obtain a lytic principle active against some other streptococcus or pneumococcus either directly or by adaptation of this phage, we accidentally obtained an organism very like our original *Streptococcus mucosus* which proved to be susceptible to our original phage. A stock guinea pig, which had been recently received from the country, died. At autopsy, which showed general thoracic involvement, we recovered from the pleural exudate, the lungs and the heart's blood a mucoid hemolytic streptococcus which was microscopically and culturally similar to our original organism. It also gave the same fermentation reactions.

This organism was readily lysed by the *Streptococcus mucosus* phage, but only when higher concentrations were used than were effective against the original organism. For example, the first titration made showed effective lysis of the guinea pig organism in a dilution of phage as high as 1 to 10,000 while the same phage was active against the rabbit organism in a concentration of 1 to 10,000,000. Subsequently we successfully obtained a phage against the guinea pig strain by cultivating it together with Madison sewage effluent. This phage was equally active against the original rabbit strain. We made no attempt to determine the antigenic identity of the two strains by the use of immune reactions but from all other points of view they were closely alike.

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[3] Kabeshima, *Compt. rend. Soc. de Biol.*, 1920, lxxxiii, 471.

3499

Improved Colorimetric Method for Determining Quantitatively the Indophenol Oxidase Content of Animal Tissues.

J. A. DYE. (Introduced by H. S. Liddell.)

From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca.

The indophenol reaction employed depends upon the oxidation of a mixture of a-naphthol and dimethyl-paraphenylene-diamine-hydrochloride, in alkaline solution, into indophenol (blue) in accordance with the following equation:

$$(CH_3)_2N.C_6H_4.NH_3Cl+CO_{10}H_7.OH+O_2=(CH_3)_2N.C_6H_4.N= C_{10}H_6O+2H_2O+HCl$$

The HCl, which prevents the reaction, is neutralized by the alkali present. This oxidation occurs spontaneously at a very slow rate when the reacting substances are exposed to air, but may be accelerated twenty times or more when minced animal tissue is added. The determinations were always carried out in open flat-bottomed Petri dishes.

The substrate in use at the present time contains 0.072 per cent of a-naphthol (M/200), 0.08624 per cent of dimethyl-paraphenylene-diamine-hydrochloride (M/200) and 0.0625 per cent sodium carbonate in 19 per cent alcohol. In order to prevent spontaneous oxidation in the substrate prior to the beginning of the oxidase test, the reagents which make up the substrate must not be mixed until needed. By having stock solutions of: (1) 0.18 per cent a-naphthol in equal parts of distilled water and 95 per cent alcohol, (2) 0.216 per cent aqueous solution of dimethyl-paraphenylene-diamine-hydrochloride, and (3) 0.3125 per cent aqueous solution of sodium carbonate, the amount of substrate needed for a test may be obtained by pipetting 2 cc. of each of solutions number one and two, and 1 cc. of the carbonate solution into the Petri dish. The success of the reaction depends upon the freshness of the diamine-hydrochloride solution which is relatively unstable and oxidizes readily in the presence of air. It is stable in the powdered form and, therefore, the solution should be prepared fresh daily.

The tissue to be studied is ground thoroughly with an equal weight of quartz sand in a mortar for exactly 10 minutes. One gram of this minced tissue-sand mixture is now accurately weighed out in a flat-bottomed Petri dish 9.5 cm. in diameter. To this is added 5 cc. of the substrate as indicated above and the contents thoroughly mixed. This may be allowed to stand for any given time interval, but should be frequently stirred or preferably rocked to insure optimum contact between tissue and substrate. Oxidation is more rapid when the tissue is merely moistened with the fluid rather than submerged. Thin films of substrate over the surface of the tissue particles are made possible by using small quantities of the substrate and flat-bottomed dishes. Vernon[1] has shown that this is not wholly a matter of better contact with atmospheric oxygen. The amount of indophenol formation induced by animal tissue is diminished when the reaction is carried out in a test tube although a continuous current of moisture-saturated oxygen was bubbling through.

After the desired period of the reaction, the contents of the Petri dish are made up to weight by adding 19 per cent alcohol. Ten cc. of 95 per cent alcohol are now added. This gives a final solution containing approximately 70 per cent alcohol. Further dilutions, when required, are made with 70 per cent alcohol so that the water-alcohol content will not vary from this point. The indophenol (blue), which is insoluble in water or the weaker alcohol of the substrate and, hence, adsorbed on the surface of the tissue particles, is dissolved by the stronger alcohol. At the same time the oxidase is inactivated, and the reaction brought to a standstill, save for a slight amount of spontaneous oxidation which continues. Fifteen minutes are allowed for the indophenol to become dissolved (under cover to prevent evaporation) and 10 minutes more for filtration. The filtrate should now be compared in a colorimeter against the standard. The time allowed for the solution of the indophenol and subsequent filtration is not so important, as the only oxidation now taking place is that which occurs spontaneously and is quite negligible. These readings, however, should be made on time when possible. Of greater importance is the time of the first dilution with alcohol which stops the reaction by inactivating the oxidase of the tissue. Even a delay of one minute may give a comparatively large error.

The standard is prepared by taking a definite measured quantity of the substrate, allowing it to stand until complete oxidation has occurred spontaneously and diluting it to 10 volumes with alcohol and water so that the final standard contains 70 per cent of alcohol. Due to the small amount of alcohol already in the substrate, 19 per cent, this is most easily accomplished by following the same procedure as used in diluting the test material, *viz.*, diluting the substrate with 2 volumes of 95 per cent alcohol and completing the dilution with 70 per cent alcohol. In order for the oxidation to be complete, a week or more is needed, but once the color has developed it remains unchanged for several months. Simultaneous development of color in the standard and unknown is quite impossible. In my experience, catalyzing agents are likely to modify the shade of color developed.

Blank tests must be run under the same conditions, with the exception of omitting the tissue, to determine the amount of spontaneous oxidation in the time of any given experiment. These readings for spontaneous oxidation should be corrected, since the available substrate diminishes as the reaction proceeds. For example, after 2 hours oxidation in the case of skeletal muscle 75 per cent of the substrate was oxidized, five per cent was the value obtained for spontaneous oxidation in a blank test for the same time. The average amount of tissue oxidation was then only $^{75-5}/_{2}$ or 35 per cent, therefore, the average amount of substrate available for spontaneous oxidation would be 100 minus 35 or 65 per cent. Now the spontaneous oxidation of the substrate without the tissue, where 100 per cent of the substrate was available for oxidation, was 5 per cent of the total, but the amount subtracted where the two are acting together should not be 5 but only 5 per cent of 65 or 3.25 per cent. Such a correction is approximate and would express the true value only when there is a linear proportionality between the time and the amount of oxidation.

As accuracy in colorimetric methods depends upon having approximately the same intensity of color in the unknown as in the standard[1], it is obvious that a single standard cannot be used after oxidation periods of unequal length. Neither is a single standard adequate when a number of tissues are being tested although the time intervals are constant. Some tissues, such as skeletal muscle, are relatively poor in indophenol oxidase while heart muscle and kidney contain a comparatively large amount. Rather than have a number of standards, this difficulty may be overcome by further diluting the test material which has already been made up to 3 volumes by adding 10 cc. of 95 per cent alcohol. The total volume of tissue-substrate-alcohol mixture is now 15 cc. and contains 70 per cent of alcohol. An additional 10 cc. of 70 per cent of alcohol maintains the alcoholic concentration, but gives a dilution of 5 times the volume of the substrate, 20 cc. a dilution of 7 times, etc. The dilution best suited to any given test can easily be determined when the per cent of complete oxidation can be anticipated in advance. This can be done with sufficient accuracy in routine work. Dilution to 3 volumes should give a perfect match where thirty per cent of oxidation has occurred, 5 volumes for fifty per cent and so on.

Calculation:

$$\frac{\text{Reading of standard. X Dilution of unknown}}{\text{Reading of unknown X Dilution of standard}} = \text{Per cent of complete oxidation of substrate.}$$

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[1] Vernon, H. M., *J. Physiol.*, 1911, xlii, 402.

3500

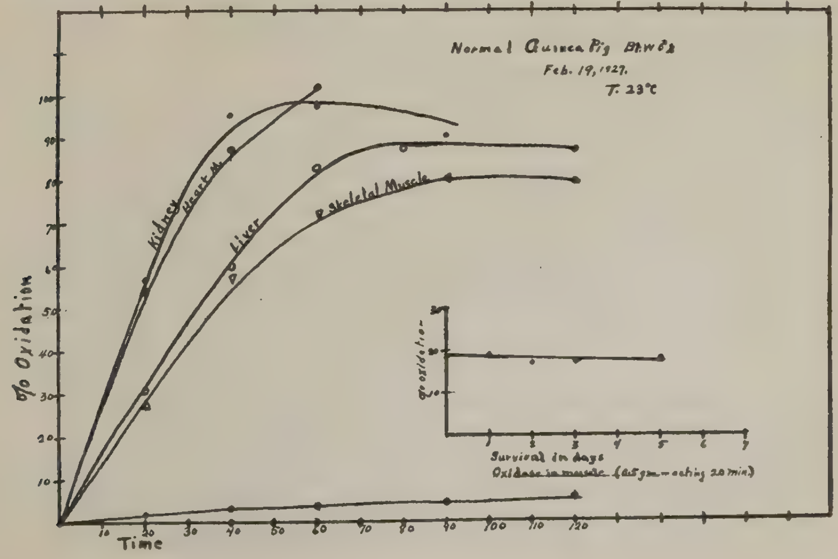
The Indophenol Oxidase Content of Normal and Scorbutic Guinea Pig's Tissues.

J. A. DYE AND R. A. WAGGENER. (Introduced by H. S. Liddell.)

From the Department of Physiology and Biochemistry, Medical College, Cornell University.

The method employed in these investigations was that reported by one of us.[1] Kidney, liver, heart and skeletal muscles were used. The last two named tissues were practically free from blood. Small amounts of connective tissues were always present, but were eliminated when possible from the test material, as they have practically no oxidizing power. The velocity of the oxidation, as indicated by the quantity of indophenol formed, was determined for tissue previously minced without sand, and tissue ground with an equal volume of quartz sand for exactly 10 minutes. In neither case were the particles of tissue more than 0.5 mm. in thickness. Determinations were made at a temperature of approximately 23° C.

The curves in the accompanying figure represent the velocity of oxidation with tissue ground with sand, and differ from those obtained from minced tissue in being steeper, and running to completion earlier. These curves have been corrected for spontaneous oxidation according to the method described.[1] In either case there is a marked difference in the power of the various tissues studied to



form indophenol. In no instance did the rate of oxidation and the time show an exact linear relationship, although in some individual experiments, particularly skeletal muscle minced without sand, an exact linear proportionality existed. In all of our experiments the curves of oxidation were almost exactly linear for a time. The deviation, which represented a diminution in the oxidation velocity, became progressively greater, until finally an equilibrium was reached. This state was not always maintained, but frequently dropped, as in the case of kidney shown in the figure. This suggests that a reductase may also be present. These observations lead us to agree with Vernon[2] that there is an excess of substrate present during the initial stages of the reaction so that an optimal contact between tissue and substrate is insured. Above this optimal there is no acceleration of the oxidation process, below it the reaction is retarded and comes quite rapidly to an equilibrium which usually lies below 100 per cent.

The oxidation of guinea pig's tissues is exceptionally stable under certain conditions. When the tissues are left in the body their power to form indophenol is scarcely altered after 5 days. An exact curve for skeletal muscle is shown in the inserted plat of the figure. Similar results were obtained for the other tissues. Alcohol and formalin inactivate the oxidase; but when the tissues are left in the body, readings may safely be made for 1 or 2 days after death.

In the comparison of the oxidizing power of normal and scorbutic animals, the readings were taken in duplicate or triplicate when the amount of tissue permitted, and were made for a period of 20 minutes only. The average error in these tests from identical material of any single animal was not more than 2 or 3 per cent. These experiments showed a marked diminution in the indophenol oxidase content of the tissues from the scorbutic animals. In those showing the most severe symptoms this reduction may be as much as 20 to 30 per cent. In general this is proportional to the severity of the disease. Quite wide variations were found between the same tissue from different animals, both normal and scorbutic; but since typical velocity curves were always obtained when any one tissue was studied and, on the other hand, duplicate or triplicate readings were made with not more than an error of 3 per cent, these variations must be interpreted by assuming that the oxidase content of these tissues differs quantitatively from animal to animal.

In the most severe cases of scurvy the body temperature was decidedly subnormal. These animals further showed a definite inability to maintain even these low temperatures when exposed to

−10° C., while in the controls the heat regulating mechanism was entirely adequate. This is a preliminary report.

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[1] Dye, J. L., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 640.

[2] Vernon, H. M., *J. Physiol.*, 1911, xlii, 402.

3501

Action of the Bacteriophage on a Low Temperature Bacterium.

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From the Department of Bacteriology, University of Illinois, Urbana.

The demonstration of the existence of a bacteriophage active at low temperatures, on a bacterium which grows well at temperatures of 0.5° C. to 4° C., is of interest in connection with the work of Koser,[1] who reported a bacteriophage active at 55° C., on a thermophilic bacillus. Since studies are in progress in this laboratory on psychrophilic bacteria from those used by Greenfield and Elder,[2] it seemed of interest to us to determine whether organisms capable of growth at low temperatures, the psychrophiles, would also be susceptible to the action of a lytic principle or bacteriophage.

The active lytic principle against this organism was obtained from raw sewage by employing the method of alternate feeding and filtration.[3] The incubation temperature during the experiment was 4° C. The lytic filtrate was found to be very active, as shown by the complete clearing of broth tubes in two days, and large lysed areas on agar slants previously inoculated with the culture. The organism which was used was a true psychrophile, since it grew well at around 4° C. There was also fairly good growth at 20° C. but no growth at all at 37° C. All of the experiments reported in this paper were carried out at 4° C. An electrical refrigerator was used for maintaining a constant low temperature. This work is being continued in connection with the studies on low temperature microorganisms.

This is a preliminary report.

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[1] Koser, S. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 109.

[2] Greenfield, R. E., Elder, A. L., McMurray, R. E., *J. Ind. and Eng. Chem.*, 1926, xviii, 1276.

[3] d'Herelle, F., The Bacteriophage and its Behavior. English translation by G. H. Smith. Williams and Wilkins, Baltimore, 1926.

3502

Preserving Supravital Staining with Neutral Red in Paraffin Sections of the Lung.

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From the Saranac Laboratory for the Study of Tuberculosis, Saranac Lake.

The method to be described is a modification of that used by McJunkin[1] which proved to be inapplicable for use on lung tissue. After our technique had been perfected Cash[2] reported a similar method, but ours, we believe, offers certain advantages. Two pathways are employed for injection of the vital stain, either the intratracheal, the intravenous or a combination of the two. In using the former the animal is first killed by air embolism. For the latter method the dye is injected into a vein 10 minutes before its death. In rabbits intravenous injections are made into the ear vein; in guinea pigs, the jugular veins are exposed under local anesthesia with 3 per cent cocaine. Ether cannot be used, as it destroys the capacity of the pulmonary cells to react specifically to the dye.

Supravital Staining—(a) Intratracheal injection: 30 to 40 cc. of a warm 1:1500 solution of neutral red made up in physiological salt solution is injected into the guinea pig's trachea, which is then tied off. The whole animal is incubated at 37° C. for 5 to 30 minutes. After this interval the dye ceases to react specifically and stains the nuclei. (b) Intravenous injection: 60 to 80 cc. of the same solution are slowly injected into the jugular vein or carotid artery of a guinea pig. If the animal survives it is allowed to live 10 minutes before being killed by air embolism. Occasionally it dies and the whole body is incubated as just described.

Fixation—This is accomplished by dropping the lungs and heart into alkaline Zenker-formol solution. After intravenous staining the lungs are distended with air. To prepare this fixative add sufficient solid NaOH to U. S. P. formaldehyde solution to give a pH value of 7.6. Dissolve by gentle heating. Keep in the incubator at 37° C. Of this alkaline formaldehyde add 15 per cent to the usual Zenker's fluid (without acetic acid). The sodium not only neutralizes the acidity, but it has a specific mordanting effect upon staining. Merk's Reagent neutral formaldehyde is not as effective as that prepared in this manner. Allow fixation to continue for 12 to 18 hours. With a razor blade cut blocks not over 2 mm. in thickness. These are washed under a vacuum in fresh Zenker's solution without formaldehyde or acetic acid. This ensures rapid removal of the formaldehyde and exhausts the air from the lung. Tissues may be used immediately or within two days.

Dehydration and Embedding—Selected blocks are washed for 15 minutes in running water and blotted on paper. They are then immersed for 10 minutes in each of the following solutions: 80 per cent alcohol, followed by graded mixtures of 95 per cent alcohol and benzene (Mark's Reagent) in the following proportions: 9:1, 8:2, 7:3, 5:5, 3:7, 2:8, and 1:9. It is often necessary, particularly with dense pathological tissues, to introduce double changes of alcohol-benzene in the dilutions of 5:5 and thereafter. The tissues are cleared for an hour or two in one or more changes of pure benzene and placed in benzene saturated with paraffin at 37° C. for an hour. Finally they are embedded in pure paraffin at 56° C. (4 changes in 30 minutes). Sections are cut, fixed to the slide and dried in the usual manner.

Counter Staining and Clearing—The paraffin may be removed from 10 or more slides at a time in a dish of xylol, but thereafter they must be treated individually, preferably by the drop bottle method. Water is more injurious than alcohol to neutral red stains. Slides are treated successively with absolute and 95 per cent alcohol and then dropped into a jar of 1 per cent iodine in 95 per cent alcohol for 15 seconds. The iodine is removed by several changes of 95 per cent alcohol.

Without washing in water they are counterstained in Harris' haematoxylin (without acetic acid) for a sufficient time to overcome the surface tension of the alcohol and to allow the dye to spread evenly on the slide. They are drained and quickly washed in 95 per cent alcohol, followed by water in a beaker, only long enough to thoroughly wet their surface. Finally the sections are dehydrated in 95 per cent and absolute alcohol, cleared in xylol and mounted in neutral balsam. Sections so prepared are clear and show deep nuclear and specific staining which is permanent and resists the action of light.

The successful application of the method depends upon the presence of an excess of dye in contact with the tissues, the slow fixation and mordanting action of the alkaline Zenker-formaldehyde solution, the rapid dehydration and clearing in the alcohol-benzene mixtures, and the use of water in counterstaining only after the excess of haematoxylin has been removed by alcohol.

The intratracheal injection of neutral red is itself an irritating process, and as such it causes the migration of many cells into the air spaces even after the death of the animal. Before employing the method to study pathological processes one should therefore familiarize himself with the reaction excited in the normal animal.

We have found the Cash method of injecting a 1 per cent solution of the dye into the veins of the living animal (10 cc. for a rabbit and 2 to 4 cc. for a guinea pig) 10 minutes before death particularly satisfactory when combined with the special methods of fixation and dehydration above described. The fixed cells in the alveolar septa are deeply stained and any stimulating effect by the dye resulting in migration of cells is avoided. However, most of the phagocytes free in the alveoli do not stain. As these are the cells of most interest in pathological processes, it would therefore seem preferable to combine intravenous with intratracheal staining, particularly where massive lesions are present.

Extrapulmonary Tissues—When neutral red is injected into the ear or jugular veins it is largely absorbed by the pulmonary tissues and the cells of other organs remain unstained. Therefore the local circulation of the spleen, liver or lymph nodes must be injected directly (20 to 30 cc. of dilute solution of dye).

Furthermore, fixation must not progress too rapidly or the dye will be dissolved. This may be overcome by placing the injected tissues in a parchment sack of neutral red solution and suspending the sack in a jar of fixative. After 30 to 40 minutes the Zenker-formol dialyses through the parchment and precipitates the dye inside the sack. The tissues are then removed and placed directly in the fixative.

In sections from organs prepared in this manner supravital staining is well preserved but the other properties of the cells naturally suffer.*

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[1] McJunkin, F. A., *Am. J. Path.*, 1925, i, 305.

[2] Cash, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 193.

* This work was begun with the assistance of Dr. Benjamin Sacks, who was unable to bring it to completion.

3503

A Phytophysiological Examination of Hemophilic Blood.

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The senior author has already called attention to the usefulness of plant test-objects in studying certain blood conditions. Thus Macht and Lubin have by means of the seedlings of *Lupinus albus* been able to make an extensive study of menstrual blood and demonstrate its toxicity for plant protoplasm.[1] Macht has reported the marked effect of the serum from patients suffering with pernicious anemia when such serum was examined for its toxicity on seedlings, so that the method could and was actually used for differential diagnosis between primary leukemia on the one hand, and secondary anemia and leukemia on the other.[2] In the present communication the authors wish to report the phytopharmacological findings obtained in three cases of hemophilia. In the case of one of these (Y) they studied the serum of a patient whose blood had already been very carefully examined by Howell and Cekada.[3] These authors found that the prothrombin, fibrinogen, calcium and the anti-coagulants which exist in the circulating blood were quite normal. They came to the conclusion that the right explanation for the delayed clotting in hemophilia is to be found in the resistant properties of the blood-platelets.

In the present investigation the tests were made on the growth or elongation of the roots of young seedlings of *Lupinus albus* by methods described elsewhere.[4] Several experiments were made with the blood of each patient, and the results of all of them were very clear-cut as compared with normal blood serum.

Patient Y, 26 years old, was one of three brothers, all suffering from hemophilia, who have been carefully studied by Prof. Howell over a period of many years. The blood picture in this case was quite normal and there were no other complications. The clotting time in this case was 2½ hours.

Patient B, is a man thirty-five years old, also showing a normal blood count, but a delayed coagulation time of about 30 minutes.

Patient K is a boy eight years old who also exhibited a normal blood picture with the exception of delayed clotting time. The coagulation time in this case was about 1½ hours. This patient exhibited some purpuric spots about his extremities.

The phytopharmacological findings were as follows. In each experiment 10 seedlings were examined for their normal growth in normal Shive solution, 10 seedlings in a 1 per cent solution of normal human blood serum, and 10 seedlings in a 1 per cent solution of hemophilic blood serum. It was found that the coefficients of growth in normal blood serum were 73, 71 and 73 per cent, which are the figures always obtained by Macht and his collaborators in other studies on blood. The coefficients of growth obtained with hemophilic serums were 74, 70 and 72 per cent respectively. In other words, the blood serum of the hemophilic subjects behaved absolutely like normal human blood serum. These findings speak against the presence of a substance which is toxic for plant protoplasm. The authors are not aware of any toxicity or hemophilic blood for animal tissues. The results, therefore, agree completely with the view of Howell and Cekada,[3] namely, that the delayed clotting of hemophilic blood is probably not due to any toxic chemical substance present in it but rather to other causes such as the greater resistance of the blood-platelets.

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[1] Macht, D. I., and Lubin, PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 208.

[2] Macht, D. I., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 209-210.

[3] Howell, W. H., and Cekada, *Am. J. Physiol.*, 1926, lxxviii, 500.

[4] Macht, D. I., and Livingston, M. B., *J. Gen. Physiol.*, 1922, iv, 573.

3504

Non-Sensitizing Protein Antigens.

W. H. MANWARING, H. D. MARINO, T. C. McCLEAVE AND T. H. BOONE.

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University.

For certain organ transplantations it has been necessary for us to find two distinct protein antigens toward which dogs may be rendered equally and practically invariably hypersensitive. We have found a very wide variation in canine susceptibility to different proteins, wholly unexpected from our experience with other animal species. The following are typical results:

(a) *Horse Serum:* Dogs injected subcutaneously with 0.5 cc. horse serum per kilogram of body weight, followed 24 hours later by an intravenous injection with the same dose, are almost invariably hypersensitive if tested after an incubation period of about 21 days. Fully 95 per cent of them are thrown into severe anaphylactic shock on intravenous injection with 1 cc. horse serum per kilogram of body weight, with fatalities in about 20 per cent of the cases. With 2 cc. horse serum per kilogram of body weight the fatalities are increased to about 40 per cent.

(b) *Goat Serum:* Dogs injected with the same doses of goat serum and tested after the same incubation period are usually not demonstrably hypersensitive. Only about 30 per cent of them give recognizable anaphylactic reactions on intravenous injection with 2 cc. goat serum per kilogram of body weight.

(c) *Egg White:* Dogs injected with the same doses of 50 per cent egg white (Ringer's solution) and tested after the same incubation period, give no suggestion of anaphylaxis, even on intravenous injection with doses as large as 5 cc. 50 per cent egg white per kilogram of body weight.

There is evidently a determining factor in canine protein sensitization of which we are at present wholly ignorant.

3505

Erythrocyte Anaphylaxis in Dogs.

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From the Laboratory of Bacteriology and Experimental Pathology, Stanford University.

Dogs injected intraperitoneally with 0.5 cc. 50 per cent horse erythrocyte suspension per kilogram of body weight followed 24 hours later by an intravenous injection with the same dose, are almost invariably hypersensitive if tested after an incubation period of about 20 days. The shock thus produced, however, differs materially from the shock in canine serum anaphylaxis.

In serum anaphylaxis, the characteristic precipitous fall in arterial blood pressure and the characteristic contraction of the urinary bladder do not begin till at least 45 to 60 seconds after commencing the intravenous protein injection. This is the time necessary for the formation or liberation of histamine-like depressor substances by the hypersensitive liver and their transmission to other parts of the body. In erythrocyte anaphylaxis (Fig. 1) both reactions begin within 15 to 20 seconds after commencing the intravenous corpuscle injection, the same time relations as those observed on intravenous injection with histamine.

It is evident, therefore, that the characteristic reactions in erythrocyte anaphylaxis do not depend upon hepatic function, but are presumably due to immediate humoral or vaso-motor reactions.

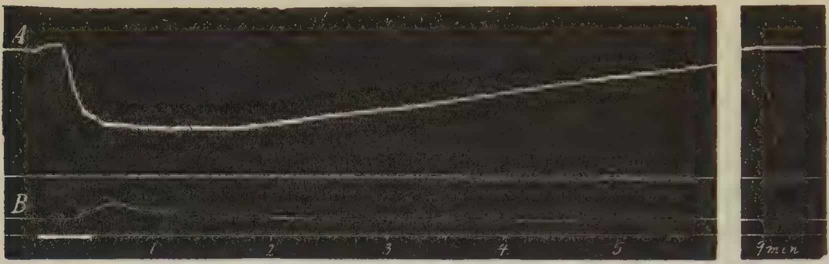

FIG. 1. *Erythrocyte Anaphylaxis in Dogs.*

A, changes in arterial blood pressure (upper base line). B, changes in the tone of the partially inflated urinary bladder, intracystic pressure (lower base line). Heavy base line, intravenous injection 2 cc. 50 per cent corpuscle suspension (Ringer's solution) per kilogram of body weight.

The above tracing represents the maximum reaction we have thus far obtained in canine erythrocyte anaphylaxis, corresponding roughly with the transcent clinical symptoms reported by Kritschewsky and Friede.[1]

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[1] Kritchewsky, I. L., and Friede, K. A., *Centralbl. f. Bakt.*, Abt. 1, 1925, xcvi, 56.

3506

Effect on Electrocardiogram of Opening Thorax and Inserting Optical Manometers into Aorta and Pulmonary Artery.

L. N. KATZ.

From the Department of Physiology, Western Reserve University School of Medicine.

Recently Weinmann and Katz[1] investigated the relation of the T wave to the asynchronous ending of right and left systole. To do this the chest was opened and optical manometers were inserted into the aorta and pulmonary artery. The objection might be raised that such procedures are sufficiently abnormal to make the electrocardiogram noticeably different, and therefore not comparable with the normal. The present report deals with the effect of such procedures on the electrocardiogram.

Meek and Wilson[2] made a comparison of the electrocardiogram in the open and closed chested dog and came to the following conclusions: "Electrocardiograms from animals with an open chest are also usually normal. By this we mean that the waves are all apparent and of the usual form. Only two variations are common. R3 is often notched and the T wave is apt to be negative or diphasic, conditions which may appear in the dog under almost any operative procedure." The present investigation substantiates these facts in general, although differing in some of the details.

In 7 dogs the standard electrocardiograms, Leads I, II and III, taken with the animal on its back under morphine and chloretone anesthesia, were compared with these three identical leads obtained after the chest was opened. A typical set of records is shown in Fig. 1. In a second series of 7 animals similar electrocardiograms,

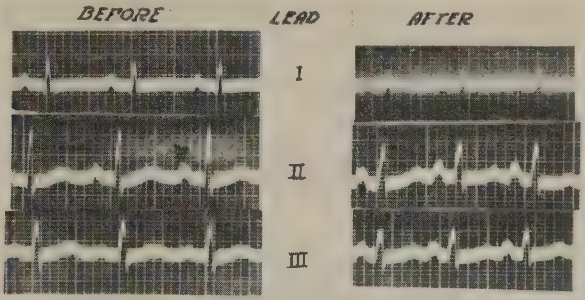

FIG. 1.

½ natural size. Standard Leads I, II and III of the electrocardiogram before and after opening chest. Time in 0.04 second.

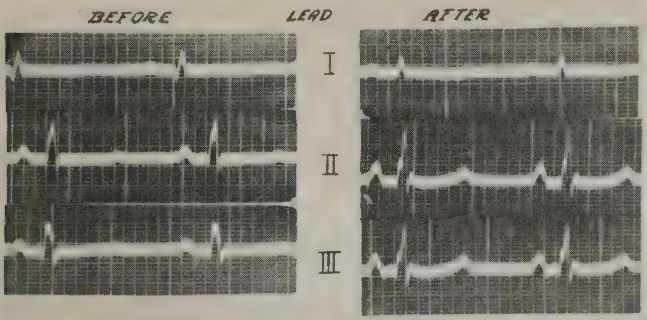

FIG. 2.

½ natural size. Standard Leads I, II and III of the electrocardiogram before and after chest opened and optical manometers inserted into the aorta and pulmonary artery. Time in 0.04 second.

Leads I, II and III, taken before the chest was opened were compared with those taken after the chest was opened and the manometers inserted into the aorta and pulmonary artery. Fig. 2 shows a typical set of such records.

An inspection of these figures reveals how little the electrocardiograms are changed. The heart rate was increased by these procedures in most cases, as in Figs. 1 and 2, but in others it remained unchanged or even decreased. No prolongation of the P-R interval was observed. The R and S deflections of the initial complex, as a rule, decreased in height in all three leads, as in Fig. 1. In several instances there was no change or, as in Fig. 2, an increase in amplitude. The splitting of R in Leads II and III of this figure occurred only in this case. The duration of QRS was not increased in any instance. In one case a decrease was observed. The changes in the gradient of the two limbs of R in Fig. 1 were the usual changes observed.

The T wave changes were not consistent. Sometimes an increase in height occurred, as in Fig. 2, and sometimes a decrease was noted, as in Fig. 1. Occasionally no change was present. The duration increased in some instances (*cf.* Fig. 2); decreased in others (*cf.* Fig. 1); or remained unchanged. On the whole, there was a tendency for the wave to become more "negative", that is, an upright T wave became inverted or its height decreased and a negative wave became deeper. There were a number of exceptions, however, such as Fig. 2. In one instance an inverted T wave became upright after the manometers had been inserted. In only two cases, one of which is shown in Fig. 2, could the changes in the electrocardiograms be ascribed to a lateral shifting in the electrical axis.

To what extent these changes can be attributed to a shifting in the electrical axis in an antero-posterior plane, to changes in the order of excitation of various fractions, to variations in the duration of electrical stress in these fractions, and to the decrease in electrical conducting material following the operation, cannot be determined at present.

The changes in any event are so small as to permit one to apply the results obtained in open chested animals with manometers in the aorta and pulmonary artery to animals not so operated.

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[1] Katz, L. N., and Weinmann, S. F., *Am. J. Physiol.*, 1927, in press.

[2] Meek, W. J., and Wilson, A., *Arch. Int. Med.*, 1925, xxxvi, 617.

3507

Studies in Experimental Thrombosis.

TAKUJI SHIONOYA,* W. R. JOHNSON AND L. G. ROWNTREE.
(Introduced by A. J. Goldforb.)

From the Division of Medicine, Mayo Clinic and the Mayo Foundation.

Pulmonary embolism is responsible for many postoperative deaths in almost every extensive surgical practice. As yet no adequate means of combating this menace has been devised and the present study was undertaken in the hope that new leads in the direction of its prevention might be forthcoming.

The apparatus used is composed of an arterial and a venous cannula connected peripherally by a transparent, semipermeable collodion tube, the whole forming a U-shaped system. The cannulas are inserted into the carotid artery and jugular vein of a rabbit after the apparatus is filled with physiologic sodium chloride solution, and the membrane portion rests in a solution of this saline. When circulation is established the blood flows through the system and back to the animal without contact with air.

The influence of many factors on the rate and type of thrombosis may be determined by means of this device. It makes it easy to vary, control, and assay mechanical, chemical, physical, pharmacologic, and pathologic factors. The process of thrombosis can be followed with the unaided eye; the membrane may be detached at any time and studied as fresh tissue or it may be embedded in paraffin and sectioned for microscopic study.

When the original technic (side tubes paraffined and cannulas with clean glass surfaces) is used with the normal animal, flow ceases through the apparatus in from 10 to 20 minutes, most often on account of obstruction of the venous cannula by white thrombi. The microscopic structure of these thrombi (and those on the membrane) is identical with that reported by Welch,[1] Aschoff,[2] and others. With circulatory and metabolic stimulants such as thyroxin and ephedrin, the flow was maintained as long as forty minutes. With single intravenous injections of heparin or hirudin, the flow persisted for from 2 to 4 hours and fibrin formation was minimal or lacking, although thrombosis proceeded through the first stages, white thrombi appearing in large quantities. These white thrombi were composed of disintegrated platelets and leukocytes only, no fibrin being recognized grossly or microscopically. Magnesium sulphate likewise retarded thrombus formation and when given in conjunction with heparin prevented the deposition of all elements, the membranes remaining free from thrombi for several hours. As would be expected, heat accelerates, and cold retards thrombus formation.

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* Rockefeller student on the Mayo Foundation.

In jaundiced animals following ligation of the common duct the flow continued for several hours without the use of anticoagulants, and the thrombi when formed were of poor quality. Intravenous injection of bile salts gave similar results; huge cauliflower-like excrescenses of white thrombi were produced which were friable and easily broken or detached.

The process of thrombosis is now being observed directly during the experiment by means of the microscope with proper illumination of the collodion membrane. Vital stains also are being employed, and these aid in differentiating the fixed from the moving elements. The technic has been changed; all the glass sections including cannulas are carefully coated with paraffin. With this modification the extracorporeal circulation persists for from four to five hours without anticoagulants when the normal animal is used. Tiny white thrombi may be seen microscopically on the membrane within ten minutes. Composed at first of platelets, they grow concentrically by the deposition of additional platelets and leukocytes and finally fibrin appears. Attempts are being made to photograph serially the process in the collodion membrane.

Other studies are being carried out. The effect of large doses of epinephrin (from 2 to 3 cc. for each kilogram) has been noted. A detailed study of coagulation factors in the presence of experimental obstructive jaundice is also under way.

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[1] Welch, W. H., Papers and Addresses, Vol. i, Pathology, 112. Johns Hopkins Press, 1920, p. 112.

[2] Aschoff, L., Lectures on Pathology, 1924. Publisher, Paul Hoeber, N. Y., 254-261.

3508

The Effect of Large Doses of Iodine on Heat Production in Rabbits.

DAVID MARINE, MAX DEUTCH AND ANNA CIPRA.

From the Division of Laboratories, Montefiore Hospital, New York City.

The administration of iodine in any form and in any manner will involute physiological hyperplasia of the thyroid, as well as prevent its occurrence in all orders of animals in which it has been studied. This includes several species of fish, for example, the Lake Erie pike, American brook trout, rainbow trout, brown trout and silver salmon; several species of birds, including pigeons, geese, chickens and turkeys, and many species of mammals including rats, guinea pigs, rabbits, cats, dogs, sheep, pigs, horses, cattle and, in man, both simple and the Graves' disease hyperplasia.

In 1921 Loewy and Zondek[1] pointed out that iodine lowered the basal metabolic rate in cases of Graves' disease, and in 1924 Plummer and Boothby[2] reported a series of 600 cases of Graves' disease in which *large* doses of Lugol's solution (10 to 15 minims) once or twice daily caused a marked drop in the respiratory exchange in most cases. Since the report of Plummer and Boothby, numerous other series have been published which confirm this fact. The idea that the lowering of the metabolic rate might be the result of the hyperplasia involuting effect of iodine was, to our knowledge, first suggested by Marine.[3] It was suggested that large doses of iodine probably cause a drop in metabolism because they bring about a rapid storage of iodine and colloid and a rapid involution of the hyperplasia. The stretching and distention of the alveoli with colloid causes partial occlusion of the peri-alveolar capillaries and lymphatics, and a pressure retention which temporarily blocks excretion until the thyroid cells have accommodated themselves to the increased pressure, and until the vascular capacity of the alveolar capillaries is restored.

In support of this view are the following facts: (1) Iodine does not lower the increased metabolism caused by feeding desiccated thyroid; (2) Iodine does not materially change the metabolic rate in individuals with fully involuted thyroids even in cases of Graves' disease; (3) Iodine is effective in lowering metabolism mainly in cases where there is a deficiency of the iodine store associated with hyperplasia; (4) Iodine, when given in very small doses (1 mg. daily), does not cause a rapid storage of colloid or a rapid fall in heat production; (5) The fall in heat production corresponds in time and probably in degree with the morphological evidence of involution of the hyperplasia; (6) Desiccated thyroid does not cause a rapid fall in metabolism even in cases with hyperplastic glands; (7) Cases with adenomatous hyperplasia which do not react to iodine with storage and involution do not show a lowering of metabolism.

Another explanation of the metabolism lowering effect of iodine in Graves' disease has been advanced by Plummer.[2] He assumes that in Graves' disease the thyroid may be secreting an abnormal product, possibly an incomplete thyroxine which iodine completes. This hypothesis is at present without supporting evidence.

With the above evidence in mind it seemed probable that the temporary fall in heat production following the administration of large doses of iodine was not specific for Graves' disease, and should occur in all animals and in all clinical associations in man, in which there is a deficient iodine store associated with thyroid hyperplasia.

As the proper human cases were not available, rabbits were used because we were equipped with the necessary apparatus and a large experience in this kind of work with this animal. The rabbit is not a very satisfactory animal, first, because it is not available for studies on basal metabolism and secondly, because marked hyperplasia of the thyroid is uncommon. Cats, likewise, are unsatisfactory because they do not develop sufficient thyroid hyperplasia. Goiterous dogs would be ideal for such studies where material is available.

With the exception of 7 rabbits which were purchased in the goiter district of northern Ohio on the chance that their thyroids might be hyperplastic, all rabbits used were reared in this laboratory. They were fed daily our regular diet of alfalfa hay and oats, with carrots or greens once a week.

The respiratory exchange measurements were begun about 15 hours after the last feeding. The standard test period was two hours, and was repeated three times each week during the control period. With the administration of iodine many of the animals were tested daily.

Eighteen received iodine either as Lugol's solution administered orally in doses of 0.075 cc. daily or as potassium iodide intraperitoneally in doses of 25 mg. daily. Three were given Lugol's solution alone (two received 8 and one 3 doses). Six were given Lugol's solution for 3 days, followed in about two weeks by potassium iodide for 3 days. This was done for the purpose of determining whether the second administration of iodine after thyroid involution had occurred would further change the metabolism. Nine were given potassium iodide alone for three days.

A summary of the results is given in the accompanying table.

After having determined the heat production for a control period of two or more weeks, a snip of the lower pole of the left thyroid lobe was removed to determine its histological condition. The iodine was then started and heat production measurements were made daily for the first few days and afterwards on alternate days for approximately two weeks. Six of the rabbits which had received Lugol's solution were given potassium iodide and their heat production measured for the next 10 days.

Results: A drop in heat production was observed in five rabbits (589b, 604, 605, 616, 622). Three of these received 0.225 cc. (about 28 mg. I) of Logul's solution and two, 75 mg. (about 57 mg. I) of potassium iodide. Two rabbits showed a slight rise in heat production. No significant change occurred in 11 rabbits, 4 of which received the above amount of Lugol's solution and 7, the above amount of potassium iodide. The drop was detectable about 24 hours after iodine administration, and lasted beyond the two weeks observation period, although tests made 2 months later showed that heat production had returned to its pre-iodine level.

As regards the relation of the thyroid condition present at the beginning of the iodine administration to the animal's reaction to iodine, nothing definitely suggestive of a relationship was found. This means very little, however, since no well marked hyperplasias occurred in any of the series. Of the five rabbits showing a decrease in metabolism the thyroids of three were classified as "early hyperplasia," while in one the thyroid was "normal". The thyroid of the fifth rabbit was not examined. Of the 11 rabbits that showed no significant change in metabolism following iodine administration, the thyroids of three were classified as "normal early," four as "early" hyperplasia, and four as "normal".

It is of interest to note that the two rabbits (581 and 589a) which received 8 daily doses of 0.075 cc. Lugol's solution showed a rise in heat production, beginning about the fifth day, and continuing until the Lugol's solution was stopped, after which it returned to normal in a few days.

It will be necessary to use rabbits with definitely hyperplastic thyroids before it can be stated that the metabolism lowering effect of large doses of iodine is or is not related to the histological condition of the gland. Martin[4] has obtained evidence that Lugol's solution in large doses lowers the metabolism in conditions other

TABLE I.

Rabbit No. and Sex	Age	No. Doses		Metab. before iodine admin. Total cal. 2 hr. periods.				Metab. after iodine admin. Total cal. 2 hr. periods.									
		Lugol's 0.075 cc.	KI).025 gm.	No. Readngs	Extremes	Mean	Average	1st day	2nd day	3rd day	4th day	7th day	8th day	9th day	10th day	11th day	13th day
581 M	Adult	8		3	11.94 10.34	10.83	11.04	10.45		12.38		12.84			13.11		12.77
589a F	284 da.	8		4	14.89 14.50	14.54	14.62	12.81		12.56		14.34	16.27		15.71		
605 M	568 da.	3		5	13.89 13.06	13.35	13.46		11.81		13.32	11.00		11.18			
589b F	574 da.	3		5	13.52 12.81	12.90	13.04	10.43	10.23	10.21	11.05			12.37		12.22	12.68
			3					11.67	11.49	11.08	11.28	11.98					
597 M	558 da.	3		5	11.93 10.96	11.45	11.49	11.00	11.76	12.31	12.43				10.50		
			3					12.16	12.26		13.06		12.71				
604 F	560 da.	3		3	13.28 12.97	13.20	13.15	12.23	13.25	11.24	11.75			11.31		11.32	10.89
			3					11.94	10.53			10.63	11.70				

TABLE I (continued).

Rabbit No. and Sex	Age	No. Doses		Metab. before iodine admin. Total cal. 2 hr. periods.				Metab. after iodine admin. Total cal. 2 hr. periods.									
		Lugol's 0.075 cc.	KI 0.025 gm.	No. Readngs	Extremes	Mean	Average	1st day	2nd day	3rd day	4th day	7th day	8th day	9th day	10th day	11th day	13th day
607 M	331 da.	3		3	14.16 13.09	13.14	13.46	13.88	12.53	12.98					12.95		
			3					13.57	11.82	13.14	13.93	13.41					
612 M	336 da.	3		3	15.47 15.18	15.33	15.33	15.18			15.22	14.33					14.68
			3					14.96		14.11	14.91	14.63	15.00				
615 F	263 da.	3		5	13.80 13.16	13.41	13.41		13.57	13.70	13.06			13.01			12.98
			3					12.98			13.59	12.77	13.49				
583 F	Adult		3	4	12.93 12.19	12.44	12.50	12.80	12.87	12.43		13.10					
609 M	330 da.		3	4	13.61 12.98	13.17	13.23		14.02		13.30		13.96				
611 M	318 da.		3	4	14.26 13.04	13.76	13.70	13.31	13.77	13.22			13.64				
616 F	Adult		3	6	14.27 12.64	13.55	13.54	11.73		10.61	11.71		11.80	11.46			
617 F	570 da.		3	5	12.76 11.12	11.43	11.85	10.90	11.39	11.44			11.07	11.49			
620 M	Adult*		3	4	11.58 11.20	11.39	11.39	10.92		11.10		10.03		12.47		12.62	11.24
622 F	Adult*		3	4	14.89 13.07	13.94	13.96	11.04	12.46	12.30	12.68	11.99		12.45			12.85
626 M	Adult*		3	4	14.58 13.24	14.04	13.99	13.06		14.37	14.33	14.86		13.92			
627 M	Adult*		3	4	14.17 13.46	14.04	13.95		13.41		14.84	14.04		13.88		14.02	

* Purchased in northern Ohio.

than Graves' disease, as for example, pernicious anemia and simple goiter.

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[2] Plummer, H. S., and Boothby, W. M., *J. Iowa State Med. Soc.*, 1924, xiv, 66.

[3] Marine, D., *Trans. Am. Climatological and Clin. Assn.*, 1925, also *Medicine*, 1927, vi, 127.

[4] Martin, K. A., (Personal communication, paper in press).

3509

Effect of Ergotamine Tartrate on the Heat Production of Normal and Thyroidectomized Rabbits.

DAVID MARINE, MAX DEUTCH AND ANNA CIPRA.

From the Division of Laboratories, Montefiore Hospital, New York City.

There are available in atropine and pilocarpine a good paralyzing and a good stimulating drug for the parasympathetic nervous system. For the sympathetic nervous system we have in adrenalin a powerful stimulant, but as yet no proven drug which paralyzes the sympathetic or neutralizes the action of adrenalin.

Recently through the work of Stoll[1] a very promising drug, ergotamine (ergotoxin of Dale[2]), has become available in sufficient amounts and purity to work with. This drug has been shown to have many activities opposite to those of adrenalin. For example, it lowers blood sugar,[3] body temperature and heat production, especially in case of exophthalmic goiter.

The only observations on the effect of ergotamine on heat production in animals which we have been able to find are those of Bouckaert,[4] who noted a rapid fall in heat production following the injection of ½ mg. of ergotamine tartrate in a thyroidized dog from +26.4 to —19 per cent. This same observer was unable to obtain a reduction in a normal dog. It seemed so unlikely that a drug which lowers metabolism in thyroidized animals should not also lower metabolism of normal animals that we were led to repeat this experiment, using rabbits and doses of 1/8, 1/4 and 1/2 mg. of ergotamine tartrate* given subcutaneously. In the control rabbits 1 cc. of 0.9 salt solution was injected subcutaneously. The results are given in the following table:

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* The ergotamine tartrate (Gynergen) was supplied by the H. A. Metz Co., Brooklyn, N. Y.

TABLE

		Ergotamine Tartrate						1 cc. 0.9 per cent Salt Solution					
Rabbit No. Sex Date of birth		612 M Born 11/20/25			617 F Born 3/31/25			589 F Born 3/12/25			607 M Born 11/20/25		
Date and amt. ergotamine inj.	Periods of 2 hrs. each	Wgt.	Total cal.	Cal. per kg. per hr.	Wgt.	Total cal.	Cal. per kg. per hr.	Wgt.	Total cal.	Cal. per kg. per hr.	Wgt.	Total cal.	Cal. per kg. per hr.
Obser. No. 1	Before inj.	gms. 2602	14.38	2.77	gm. 2728	11.90	2.18	gm. 2582	10.57	2.05	gm. 2465	12.46	2.53
12/7/26	After inj.												
1/8 mg.	1st		13.87	2.67		11.61	2.14		12.10	2.35		12.62	2.56
	2nd		12.18	2.35		11.72	2.16		11.70	2.27		14.64	2.98
Obser. No. 2	Before inj.	2496	12.52	2.51	2621	11.78	2.25	2463	**	**	2420	11.06	2.28
12/10/26	After inj.												
1/4 mg.	1st		11.79	2.38		9.88	1.88		12.10	2.46		12.17	2.52
	2nd		10.68	2.16		10.24	1.96		11.84	2.41		12.40	2.57
	3rd		10.88	2.20		8.90	1.70		10.59	2.16		11.62	2.41
	4th		11.22	2.27		11.23	2.15		10.70	2.18		11.18	2.32
Obser. No. 3	Before inj.	2531	13.01	2.57	2605	10.89	2.09	2510	13.21	2.63	2372	12.00	2.53
12/15/26	After inj.												
1/4 mg.	1st		11.12	2.20		8.83	1.70		12.46	2.50		12.19	2.57
	2nd		11.46	2.27		9.12	1.75		12.87	2.60		10.85	2.29
	3rd		11.67	2.31		10.54	2.03		12.76	2.57		11.20	2.37
	4th		12.04	2.39		10.69	2.06		11.96	2.42		11.07	2.35
Obser. No. 4	Before inj.	2598	14.58	2.81	2595	10.22	1.96	2597	**	**	2394	13.01	2.72
12/22/26	After inj.												
1/2 mg.	1st		11.50	2.22		8.37	1.62		13.66	2.66		13.59	2.85
	2nd		12.42	2.40		9.07	1.75		13.81	2.69		11.02	2.31
Obser. No. 5*	Before inj.	2839	11.02	1.94	2822	9.61	1.70	Died of pneumonia 12/24/26			2445	9.65	1.97
1/6/27	After inj.												
1/4 mg.	1st		10.97	1.94		8.24	1.46					10.21	2.09
	2nd		10.65	1.88		9.53	1.69					10.38	2.13

**Lost.

*Thyroidectomy 12/23/26.

Ergotamine tartrate in the doses used causes a striking fall in heat production in normal rabbits without the occurrence of detectable untoward effects. The metabolism lowering effect of ergotamine tartrate was evident within an hour after its injection (the exact time of onset was not determined as our shortest observation period was 1 hour) and may last more than 8 hours. The fall in metabolism was proportional to the doses of the drug used. These rabbits were thyroidectomized, and after allowing 14 days for the metabolism to fall to the myxedema level, a single observation was made on three of them, using a dose of 1/4 mg. of ergotamine. This observation suggests that ergotamine also lowers the metabolism in the absence of most of the thyroid gland, though to a much less extent than in normal animals.

These experiments offer additional proof that ergotamine is a powerful sympathetic depressant and should be of value in the further study of the physiology and pathology of the vegetative nervous system. Ganter[5] has spoken of ergotamine as the "Atropine of the sympathetic." Ergotamine has already been extensively used in the treatment of exophthalmic goiter, as shown by the reports of Bouckaert,[4] Kliment,[6] Merke,[7] Ruetz,[8] Schönbauer,[9] Adlersberg and Porges,[10] Novons and Bouckaert,[11] and others. The reason for its trial in Graves' disease is obvious, *viz.,* that sympathetic stimulation, or at least loss of inhibitory control, is the outstanding physiological feature of the disease.

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[1] Stoll, A., *Verhandl. der Schweiz. Naturf. Ges.*, 1920, S. 190.

[2] Dale, H. H., *J. Physiol.*, 1906, xxxiv, 163.

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[6] Kliment, E., *Casopis lekaru ceskych.*, 1925, lxiv, 1417; Abst., *J. Am. Med. Assn.*, 1925, lxxxv, 1507.

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[9] Schönbauer, L., *Deutsch. Z. f. Chir.*, 1926, cxcviii, 99.

[10] Adlersberg, D., und Porges, O., *Klin. Wchns.*, 1925, iv, 1489..

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3510

On Feeding Certain Liver Constituents in Pernicious Anemia.*

RANDOLPH WEST.

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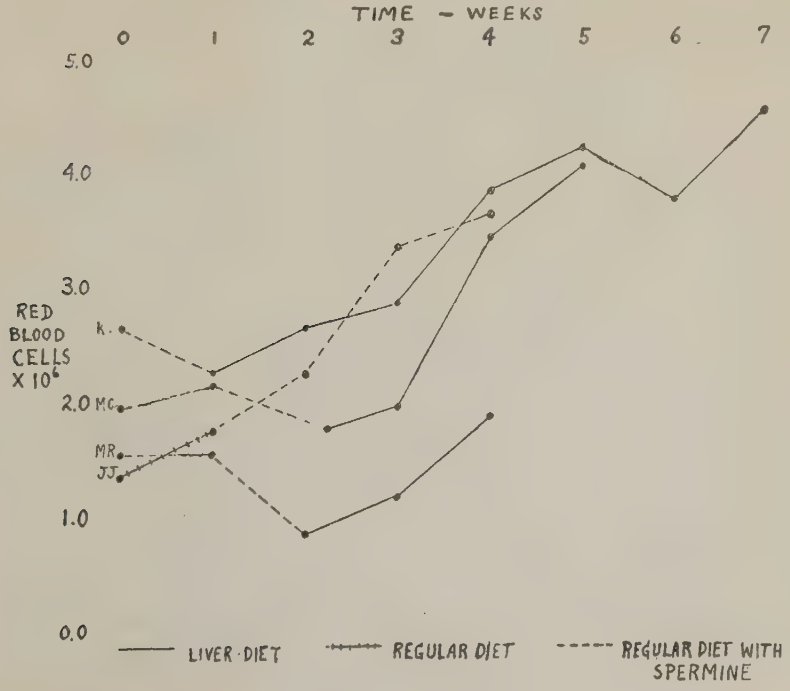
Since Minot's[1] demonstration that a high liver diet is extraordinarily useful in the treatment of pernicious anemia we have been attempting to find whether certain liver constituents can be used as effectively as whole liver.

Spermine was prepared from beef pancreas by the method of Dudley[2] by steam distillation and precipitation as the phosphate. The phosphate was dissolved in weak acetic acid and fed in this solution, 100 to 150 mgm. daily being added to a diet containing no liver, kidney nor sweetbread. The distribution of spermine suggested its trial, for 100 gm. of tissue semen contains 260 mgm., pancreas 25, liver and kidney each 15, muscle and thymus 5.

The clinical results were believed to be negative and are appended. (Fig. I.) Case J. J. was probably in a period of spontaneous improvement.

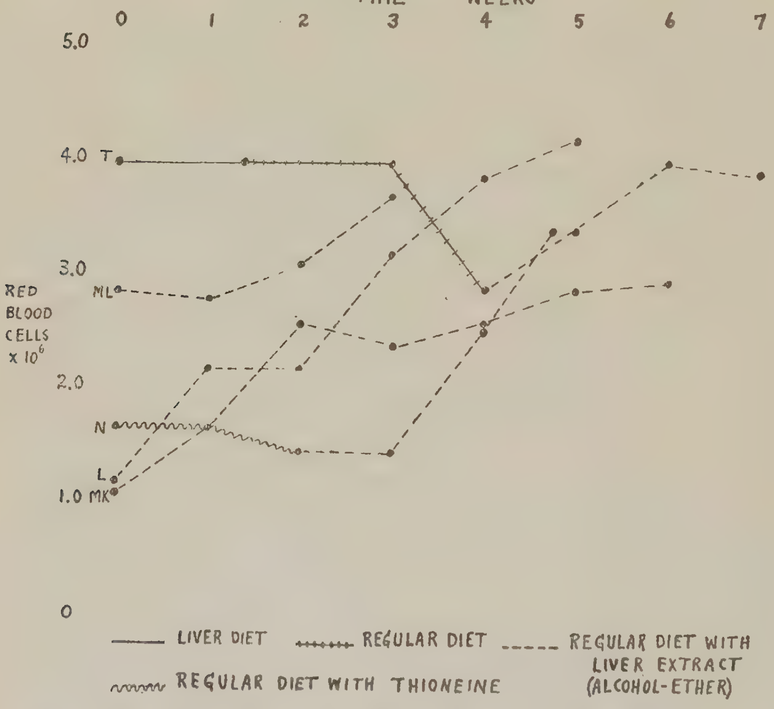
FIGURE I



The alcohol ether soluble fractions of fresh beef liver were used next. Five kilos of liver were obtained fresh from the slaughter house, minced, twice extracted with 4 liters of cold 95 per cent alcohol, then 2 to 3 liters of cold ether, and finally 4 liters of boiling alcohol. The three fractions were filtered separately through filter paper and evaporated almost to dryness *in vacuo*. The yield was 360 to 420 gm. (about 30 per cent water) and 10 to 15 gm. were emulsified with grape juice and fed twice daily.

Five consecutive cases have shown improvement on this regime, as shown on the appended chart. (Fig. II.)

FIGURE II



Finally, one patient has been placed on 50 to 100 mgm. thioneine daily. At the end of 12 days he was definitely worse; thioneine was replaced by the liver extract and he has improved.

The alcohol ether soluble fraction has been divided by washing several times with 95 per cent alcohol, then ether, then 95 per cent alcohol again. The residue gives a turbid solution in water and 70 cc. a day has proven active in one case. Per 100 cc. this solution contains 13.8 gm. solids. The solids show no iron, negative buiret, 5.7 per cent N, 2.4 per cent NH_2—N, 2.0 per cent P, 1 per cent S.

This is a preliminary report.

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[2] Dudley, H. W., Rosenheim, M. C., and Rosenheim, O., *Biochem. J.*, 1924, xviii, 1266.

[3] Newton, E. B., Benedict, S. R., and Dakin, H. D., *J. Biol. Chem.*, 1927, lxxii, 367.

3511

The Metabolism of Acetic Acid in Phlorhizin Diabetes.

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From the Department of Physiology, Cornell University Medical College, New York City.

Geelmuyden[1] has stated that acetic acid is convertible into glucose, as demonstrated by the production of extra sugar after the subcutaneous injection of the sodium salt into phlorhizinized dogs. He therefore assumes that it is an intermediary product in the hypothetical conversion of fat to carbohydrate. This investigator fails to call attention to an experiment of Ringer and Lusk[2] in which the oral administration of 10 grams of sodium acetate failed to elicit any additional sugar formation in a phlorhizinized dog.

The question was of sufficient interest to warrant the carrying out of additional experiments. We have been unable to give large doses of sodium acetate by mouth on account of its non-absorption, due to the violent catharsis which it produces, although in the experiment of Ringer and Lusk absorption was evidently complete. Three experiments have been made in which the substance was introduced intraperitoneally in amounts as great as 27 grams, and six tests in which it was injected subcutaneously. There was no indication from these results on completely phlorhizinized animals of any tendency of this substance to form glucose.

The excretion of volatile acids in the urine increases somewhat after the administration of the sodium acetate, but not sufficiently to account for any considerable percentage of the acetic acid injected. The conclusion is that acetic acid is not convertible into glucose nor largely excreted as such by a phlorhizinized dog. It must be largely oxidized. The hypothesis that fat may be transformed into glucose based on such evidence is, therefore, not valid.

This is a preliminary report.

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[1] Geelmuyden, H. C., *Ergeb. d. Physiol.*, 1923, xxii, 51.

[2] Ringer, A. I., and Lusk, G., *Z. f. physiol. Chem.*, 1910, lxvi, 106.

3512

pH Concentration of Intestinal Contents of Dog, with Special Reference to Inorganic Metabolism.

DAVID M. GRAYZEL AND EDGAR G. MILLER, JR.
(Introduced by Wm. J. Gies.)

From the Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons.

Ten dogs were fed a normal diet (125 gm. meat, 100 gm. cracker meal, 30 gm. bone ash, 15 gm. lard, 300 cc. water); 3 dogs were fed a diet comparatively high in carbohydrate (25 gm. meat, 150 gm. cracker meal, 30 gm. bone ash, 30 gm. lard, 350 cc. water); 2 dogs were fed a high protein diet (300 gm. meat); and 2 a diet high in fat (50 gm. meat, 50 gm. cracker meal, 30 gm. bone ash, 180 gm. lard).

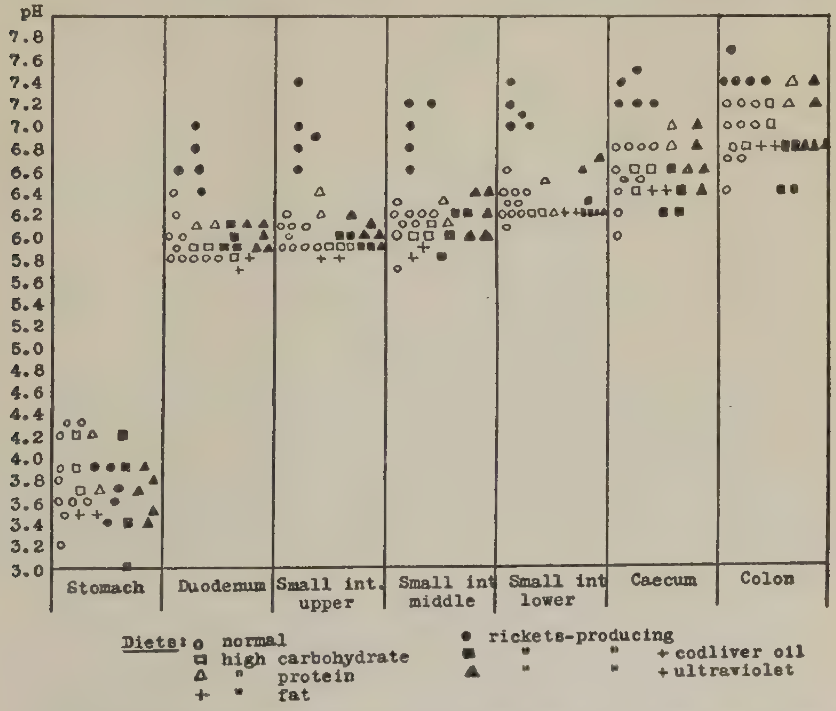
Fifteen dogs were fed a diet which has been shown by Mellanby[3] to produce rickets in young dogs (175 cc. milk, 200 gm. white bread, 2 gm. NaCl). Of these, 4 were also fed 10 cc. of cod-liver oil daily; and 5 were irradiated with ultra-violet light for periods ranging from 40 min. to 3 exposures of 45 min. each on consecutive days. Four to six hours after the last feeding the dogs were killed by intravenous injection of amytal, and the alimentary canal removed. The contents of the stomach, duodenum, 2 ft. sections of the intestine, the caecum, and the colon were obtained and the pH determined.

112 determinations (15 dogs) were made both electrometrically and colorimetrically. In the other determinations the colorimetric method was used. Comparison of the two methods justifies reliance on comparative results as obtained colorimetrically. The average error of the colorimetric method, as compared with the electrometric, was 0.2 of a pH unit; the maximum difference was 0.39, and the minimum 0.1; in every case the error was minus.

The average acidities, in terms of pH, are shown in the table. The variations are indicated in the chart.

TABLE I.

No. of dogs.	Diets	Stomach	Duod.	Upper small int.	Middle small int.	Lower small int.	Caecum	Colon
10	Normal diet	3.68	5.91	5.99	6.27	6.36	6.57	6.84
3	High carboh. diet	3.93	5.87	5.90	6.03	6.20	6.53	7.00
2	High fat diet	3.50	5.75	5.80	5.85	6.20	6.40	6.80
2	High protein diet	3.95	6.00	6.30	6.23	6.45	6.90	7.30
6	Rachitic diet	3.71	6.59	6.81	6.88	7.05	7.27	7.44
4	R. + C. L. O. diet	3.40	5.97	5.92	6.02	6.11	6.32	6.52
5	R. + Irradiation	3.62	5.99	5.94	6.16	6.26	6.63	6.94



The normal diet showed acidity throughout the tract, with considerable constancy at each level. High fat, protein, or carbohydrate variations caused no notable changes, the results falling within the normal ranges.

The rickets-producing diet caused a definite rise in the pH of the contents of the intestines, even to alkalinity. Ultra-violet irradiation or cod-liver oil administration (both of which are known to be curative or preventive for rickets) to dogs on the same diet caused an acidification of the intestinal contents to the normal range. The gastric contents showed no changes.

The contents of the small intestines of rats, and the feces of rats, have been shown to have a pH above normal on a rachitic regime of the low phosphorus type, while curative procedures reduce the pH to normal.[1, 2, 7] There is evidence that cod-liver oil, ultra-violet light, or the ingestion of acid or alkali, affect the degree of absorption of phosphate (and calcium) from the intestine.[4, 5, 6] That the primary defect in rickets is an impairment of absorption has been argued before. These experiments add evidence for this view. The pH of the intestinal contents, with the consequent effect on the solubility of phosphate (and also of calcium, magnesium, and iron) offers a mechanism whereby the effect of such agents as light and cod-liver oil may be linked with the inorganic metabolism.

The addition of 10 gm. of calcium lactate (2 dogs) or of dibasic calcium phosphate (2 dogs) to the rickets-producing diet was also found to give the same intestinal pH values as did the normal diet. The relation of this to possible anti-rachitic effects, and the mechanism whereby the pH is controlled physiologically, are subjects for further research.

This is a preliminary report.

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3513

Biochemical Studies of Human Semen and the Mucin of the Cervix Uteri. I.

R. KURZROK AND E. G. MILLER, JR. (Introduced by Wm. J. Gies.)

From the Biochemical Laboratory of Columbia University at the College of Physicians and Surgeons.

The plug of mucous filling the cervical canal and the external os is very viscous and adhesive, and when pulled away from the cervix it forms a slimy string which is broken with difficulty. In cases presenting no pelvic pathology the mucous is semi-transparent; when there is a lesion such as an infection, laceration, eversion, or erosion, the cervical mucous tends to become muco-purulent in character and more adhesive. The mucous plug consists very largely of a mucin and water. It presents, unless altered, a very considerable barrier to the passage of spermatozoa.

When a shred of mucous is placed in a test tube with semen and incubated, within 30 min. it becomes dull in appearance and the edges of the shred are frayed and fragmenting; in 12 to 24 hours the shred has entirely disappeared. In control tubes, containing no semen, the mucous shred remains intact and does not change in appearance. This lytic action is influenced by pH. Using phosphate buffers, maximum activity was found to occur in two ranges, pH 5.28 to 5.90 and 7.37 to 8.04. Between these optima the action is less; beyond them, also less; below 4.0 there is no action; above 10 equal digestion occurs in both the control tubes and the tubes containing semen, suggesting a solvent action due to alkalinity.

The lytic action seems to be specific to a high degree. Semen will not digest shreds of fibrin, egg white, or casein, either in acid or alkaline medium; it was found to have no effect on small masses of respiratory or salivary mucins. The shreds of cervical mucin are highly resistant to pepsin and trypsin.

The substance responsible for the action is thermolabile; it can be precipitated by phosphomolybdic acid or alcohol; it does not dialyze through a collodion membrane. It seems not to deteriorate during five days standing in the cold. The facts suggest that the solvent action of semen on a mass of cervical mucin is enzymic.

In clinical observation, the lytic action of the semen on the mucous is seen to occur *in vivo* after coitus. The presence of the lytic substance does not depend on the presence of spermatozoa. A specimen of semen containing no spermatazoa dissolved the mucous. Semen from a case of Froelich's Syndrome, containing a few dead spermatozoa, was inactive. A sample containing a normal number of motile spermatozoa was found to be inactive (this case was the husband in a sterile marriage, the wife being normal).

Tests made with mucous from a patient with a leucorrheal discharge due to a lacerated cervix, and from one with acute gonorrhea, indicated that the digesting action of normal semen is markedly diminished or stopped by the presence of pus in the mucous.

The experiments described suggest that the lytic substance may be an important factor in the passage of spermatozoa up the tract, and that its absence may be an etiological factor in some cases of sterility in which no explanation has hitherto been offered.

We were unable to extract any of the lytic material from the testis of a bull. The origin may be in the testis, prostate, or seminal vesicle.

Semen strongly reduces thionin to a colorless compound. The material responsible for this reduction is thermostabile, dialyzable through collodion, and is carried down by precipitation with phosphomolybdic acid, alcohol, or std. ammonium sulphate.

Semen, with guaiac or benzidine, shows no indication of oxidase or peroxidase. It contains catalase. It does not contain glutathione. It is very strongly buffered.

This is a preliminary report.

3514

Weight of Mouse Embryos 10-18 Days After Conception, a Logarithmic Function of Embryo Age.

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From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island.

The following methods were adopted in the endeavor to provide the optimum developmental conditions and to reduce the variability of the mouse embryos as far as possible. The mothers were self-colored, intense, brown-agoutis, F_1 hybrids between two highly inbred, pedigreed strains, the females from the Bagg albino and the males from the Storrs-Little, characterized by pink-eyed dilute brown self-color. The albino grandmothers at conception were over ten weeks old and had not nursed young for at least three weeks; the mothers were nursed in litters that had been cut down to six at birth; they were weaned at four weeks and held in large mating boxes, not more than six to a box, until over three months old before mating was first permitted. The fathers of the embryos weighed came from the inbred line 89,-self-colored, intense, brown-agouti; they were kept individually in small boxes. This type of mating gives embryos that may be called triple hybrids; they bear the maximum heterozygosity and hence show the minimum amount of segregation. When matings were desired each male was placed with the females in a certain box for one hour and the females then examined for vaginal plugs as evidence of copulation. Each female with a plug was immediately given a small box to herself until the hour assigned for weighing the embryos; she was killed at some exact multiple of 24 hours from the end of the hour she was with the male.

The membranes and placentae were removed with the aid of a binocular microscope; for embryos of 10, 11 and 12 days this was done in Locke's solution. As soon as a dissection was finished the excess fluid was blotted off and the embryo placed in a glass ring of appropriate size between cover glasses, or in a small weighing bottle (18-day embryos), and carried at once to the balance pan. While one embryo was being weighed the next was dissected. A Sartorious balance of 1/10 mg. sensitivity was used and the weights recorded with four figures of three significant digits. The original records for the fourth place are in the form of pointer readings.

TABLE I.

Conception age t	Embryo age (t-7)	Frequencies		Average weight	
		Litters	Embryos	Observed Σ W/n	Calculated $W = .000188\ (t\text{-}7)^{3.656}$
10	3	7	59	.0086	.0104
11	4	10	101	.0329	.0299
12	5	11	77	.0762	.0675
13	6	11	100	.1298	.1315
14	7	11	74	.2288	.2311
15	8	10	75	.3651	.3766
16	9	12	95	.5926	.5793
17	10	11	89	.8467	.8515
18	11	10	91	1.190	1.206

The table gives the number of litters and the number of embryos of each age weighed. The averages give each litter weighted by the number of individuals, (the sum of the individual weights over the total number of embryos). Plotted on uniform coordinate paper, these averages form a fairly smooth curve; plotted on semi-logarithmic paper they give a strongly curved line, which shows that this series can not be expressed by an exponential equation. The graph obtained on double logarithmic paper is also a curved line. By shifting the zero point for age to various positions it was found that a close approximation to a straight line was obtained on double logarithmic paper when age t was changed to t-7. This indicates that during the period of observation the weight of the embryo is a logarithmic function of its age, if the *embryo age* is given as 7 days less than conception age. The equation for this curve is the power function of the form, $W = Kt^n$, in which W is the weight, t the age, n the slope of the graph on double logarithmic paper and

K the intercept on the W axis, that is, the weight at the end of the first day. The constants obtained when substituted in this equation in logarithmic form give: $\log W = 3.656 \log (t-7) + \log .000188$.

Since the first stages of development of a mammal consist of the formation of the pro-embryo, a considerable period elapses before the first organization of the embryo proper. This is the justification for assuming embryo age to be less than conception age. In the mouse the first differentiation of the embryo proper (primitive streak) is not found before the end of the first week. Thus the embryological evidence bears out the purely graphical result obtained by shifting the age (t-7) until the embryo weights fit a logarithmic straight line. Data for younger embryos are being collected.

The mouse embryo at seven days after conception and the chick embryo at the beginning of incubation are in practically the same stage of development. Starting the age scale at these points, the data for both animals lie on straight lines on double logarithmic paper, whose slopes are practically identical (chick, $n = 3.6$, Murray[1]; mouse, $n = 3.656$) though the intercept on the W axis is considerably higher for the chick ($K = .665$). These constants seem to indicate that the mouse embryo starts out with less material than the chick, but at the same age the rate of growth in both cases is practically the same. Handled graphically as above the prenatal weight data for guinea pig (Draper[2]), rat (Stotsenberg[3]), and man (Streeter[4]) appear to give fair indications of the time when the respective primitive streak stages are reached, and support the conclusion that the growth of the pro-embryo requires a different equation from that of the embryo. In studying the embryo, embryo age instead of conception age provides a more accurate basis for comparison.

This is a preliminary report.

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3515

Pathogenic Anaerobic Bacillus Not Hitherto Described Cultured From Fatal Operative Wound Infection.

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This organism was cultured post-mortem from the lesion in an operative wound infection. The bacteriophagic study was carried out by two of us (F. L. M. and F. B. H.). The patient was operated on and cared for in another hospital by L. C.

The infection began on the eighth day and caused death on the thirty-fifth day after operation. The only other organism found was *B. pyocyaneus.* The virulence for laboratory animals of the organism to be described tends to confirm the belief that this organism was the cause of the human lesion and is pathogenic for man.

Pathogenicity. It is lethal in small doses for white mice, white rats, guinea pigs, rabbits, pigeons, chickens, cats and dogs. No other animals were tested. In these animals, when injected subcutaneously, it produces an extensive hemorrhagic edema resembling the lesions produced by *C. novyi (B. oedematiens)* and *C. oedematis maligni (Vibrion septique),* but differing somewhat from each of these. The animals succumb to the infection within a few hours or days. The organism may be recovered after death from the lesion, the peritoneum and the heart's blood. The pathogenicity of the strain has been maintained for nearly two years with repeated transplantation on artificial media.

Morphology. It is a gram positive bacillus varying from 1 to 4 mu in length and 0.3 to 0.5 mu in thickness. It is motile in young cultures. It produces spores readily on all media except those containing dextrose. The spores are generally para-central, but many are found free. The vegetative forms have no capsule, but the spores appear to have a thin capsule with the Hiss stain.

Anaerobiosis. Conditions of anaerobiosis for this study have been accomplished by the use of a modification of the McIntosh and Filde jar. The organism requires the same degree of anaerobiosis as *C. welchii.*

Growth on Solid Medium. The solid medium used has been chiefly sheep's blood agar in Petri plates. On this medium discrete stellate colonies are formed which are gray in color and very irregular in outline. Occasionally there is a fernlike spread from one side of the colony. At first it was thought that this represented a contamination, but such was not the case. There is no hemolysis, but when the colony is scraped off there is slight pallor underneath. Occasionally there is a faint green tinge to the margin of the colony. The growth has a cheezy but not a butyric odor. In deep agar it produces small discrete mossy colonies. In dextrose agar large quantities of gas are formed.

Growth of Fluid Medium. On plain meat infusion broth it grows readily with a mucoid, ropey consistency. On cooked meat medium this quality is less marked at first, but is present in older cultures. The growth is diffuse and on long standing gradually settles to the bottom of the fluid portion of the medium. Gelatin is completely liquified in forty-eight hours.

Fermentation Tests. It is not an active fermenter. Gas and acid are formed with dextrose, but not with lactose, saccharose, salicin, mannite or glycerin. Milk is not acidified except after prolonged incubation.

Proteolytic Tests. There is very slight erosion after long incubation on Loeffler's medium but no real liquifaction. The casein of milk shows very slight digestion after long incubation. The surface of the meat in cooked meat medium becomes dark in old cultures.

Toxin Production. It produces a true exotoxin. This toxin is filterable, thermolabile, has a latent period and is capable of producing an antitoxin when injected into rabbits, either subcutaneously or intra-venously, in sublethal doses. Of this immune rabbit serum 0.05 cc. will protect white mice against 0.45 cc. of toxin. This toxin is produced both in plain broth and in 0.2 per cent dextrose cooked meat medium. The minimal lethal dose of the toxin for 20 gram white mice is 0.02 cc. when injected subcutaneously.

The effect of B. oedematiens and Vibrion septique antitoxin. Potent *B. oedematiens* anti-serum, of which 0.05 cc. will protect white mice against 0.45 cc. of toxin produced by a typical strain of *B. oedematiens,* the M. L. D. of which is less than 0.05 cc., has no effect whatsoever on a minimal lethal dose of toxin produced by this organism. The same is true for potent *Vibrion septique* antitoxin.

The effect of antitoxin for this organism on the toxin or the centrifuged supernatant fluid of a culture typical strains of B. oedematiens or Vibrion septique. Rabbit antiserum which is potent in protecting white mice against this toxin to the degree mentioned above and against infection with the centrifuged supernatant fluid of a culture of this organism will not protect mice against a minimal lethal dose of *B. oedematiens* toxin, or *Vibrion septique* toxin or against infection with these organisms.

Two other strains exactly similar to this have been recovered from two out of four tubes of catgut from a batch used in the hospital at the time this infection developed.

Summary. An anaerobic bacillus has been cultured from the lesion in a fatal human operative wound infection and from catgut. It is pathogenic in small doses for eight different species of laboratory animals. It produces a lesion in these animals resembling somewhat the lesions produced by *B. oedematiens* and *Vibrion septique.* It differs from these organisms strikingly in certain of its cultural characteristics. Serologically it has been demonstrated to be distinct from both of these. As far as we know it does not correspond to any other pathogenic anaerobe heretofore described. The name proposed for this new species is *Clostridium oedematoides.*

This is a preliminary report.

3516

General and Local Heat Developed in Living Animal Body by Passage of High Frequency Currents.

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From the Hospital of the Rockefeller Institute for Medical Research.

The present wide use of clinical diathermy has led us to a study of the deep heat produced in tissues by the passage of alternating currents of high frequency. Most of our experiments have been made on dogs anesthetized by the intravenous injection of barbital sodium. The deep temperature was measured by means of specially prepared thermocouples soldered to the tips of Luer needles which could be inserted into the body cavities. These thermocouples, which were of copper and constantan, were connected with a galvanometer through a constant temperature junction obtained by a thermos bottle thermostat as described by Clark.[1]

The high frequency current was passed through the body by placing the electrodes usually on the sides of the shaved thorax. Great care was taken to have the electrodes parallel to each other and the thermocouple needles at right angles to the electrical field. This is very important, otherwise there is a concentration of current at the needle point which results in false readings. This source of error was excluded in all experiments. In some instances the temperature was measured by mercury thermometers as well. The current strength usually employed was between 1200 and 1500 milliamperes, with a concentration not greater than 100 milliamperes per square inch of electrode surface. Under these conditions no damage to the skin resulted.

The passage of such a current through a dog's thorax results in a general elevation of body temperature (rectal), with an equivalent rise of temperature in the lung. This is shown in Fig. 1. In this figure it is likewise shown that with the passage of the current the lung at first gradually warms more than the rectum, but later cools below the rectal temperature until the current strength is increased, when it again crosses the rectal temperature.

Fig. 2 shows the effect of sudden death on the temperature recorded by a mercury thermometer placed in a dog's abdomen between electrodes. The sudden precipitous rise indicates that the cooling mechanism at work during life has suddenly ceased to operate.

That this cooling mechanism might be related to surface temperature is brought out in Fig. 3. But that it is far more likely depen-

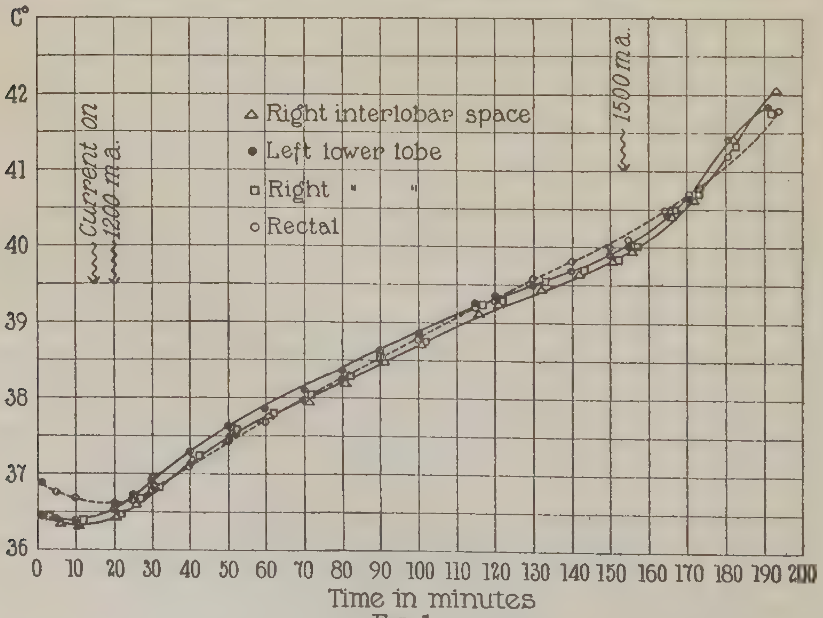

FIG. 1.

Temperature recorded by thermocouples in lungs and thermometer in rectum, of anaesthetized dog, subjected to high frequency current.

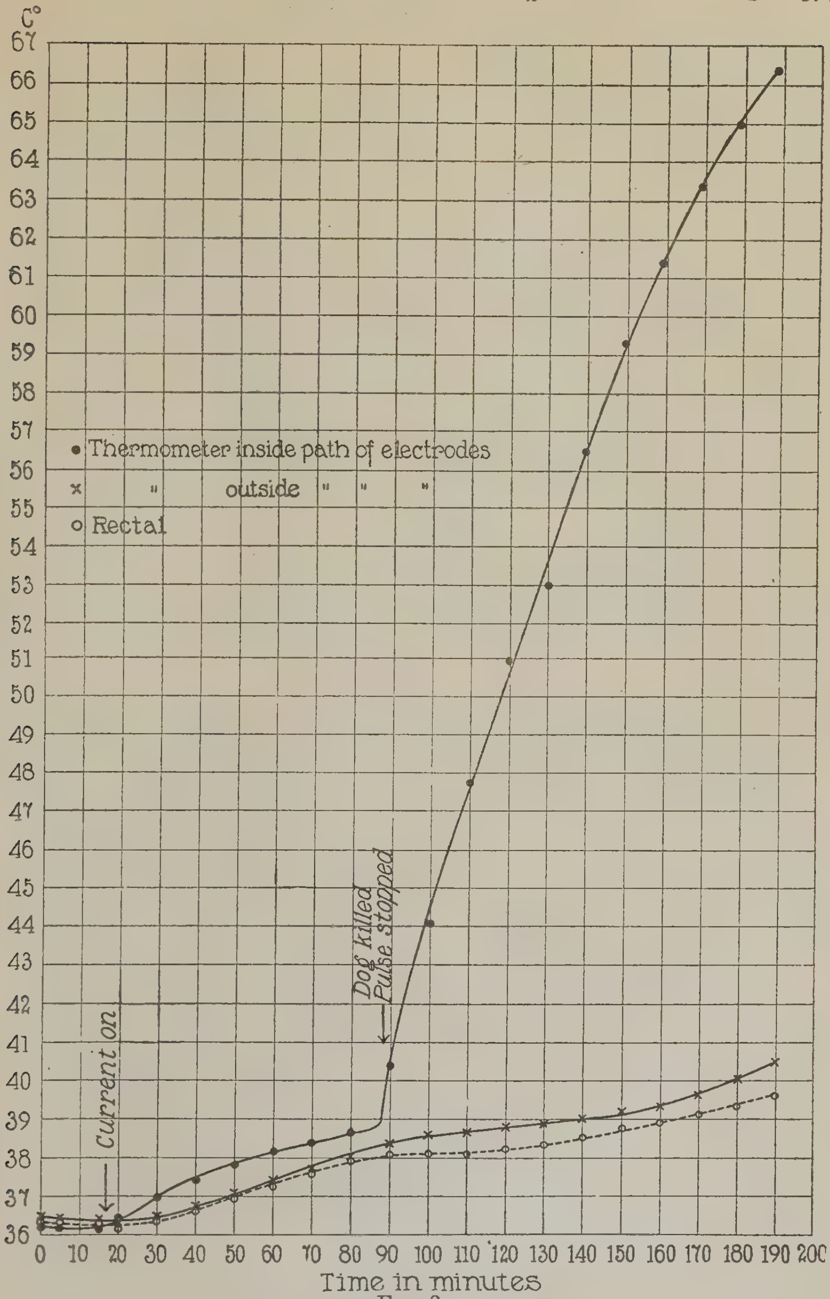

FIG. 2.

Effect of death on heat recorded by thermometer, placed in abdomen between the electrodes.

dent upon normal local circulation is shown, we believe, clearly in Fig. 4 and Fig. 5.

In the experiment summarized in Fig. 4, the left branch of the pulmonary artery was surrounded by a pneumatic cuff, as described by Moore and Binger,[2] in such a manner that the circulation through the artery could be obstructed or released at will while the animal was breathing with closed thorax. Obstruction to the circulation in the left lung was immediately followed by a local rise in temperature, which fell promptly when circulation was again reestablished. Subsequent sudden death of the animal resulted in a still more precipitous rise of temperatures in both lungs. That this added rise is due to the cessation of the bronchial circulation is indicated in Fig. 5, where the "death curve" was obtained in one lung as soon as its root was ligated. A control experiment showed that ligation of the bronchus alone, without the vessels, had no such effect.

This work represents a preliminary report on a critical study of diathermy. It has led to some interesting observations on heat distribution. At present experiments are being made on temperature measurement of the circulating blood at various points in the animal body.

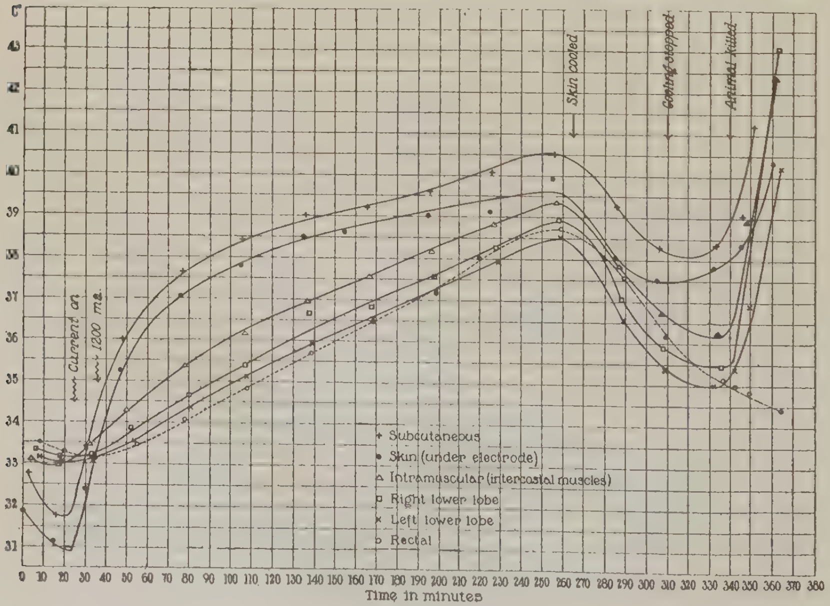

FIG. 3.

Effect of cooling the skin, during passage of high frequency current, on temperature recorded by thermocouples placed at various points.

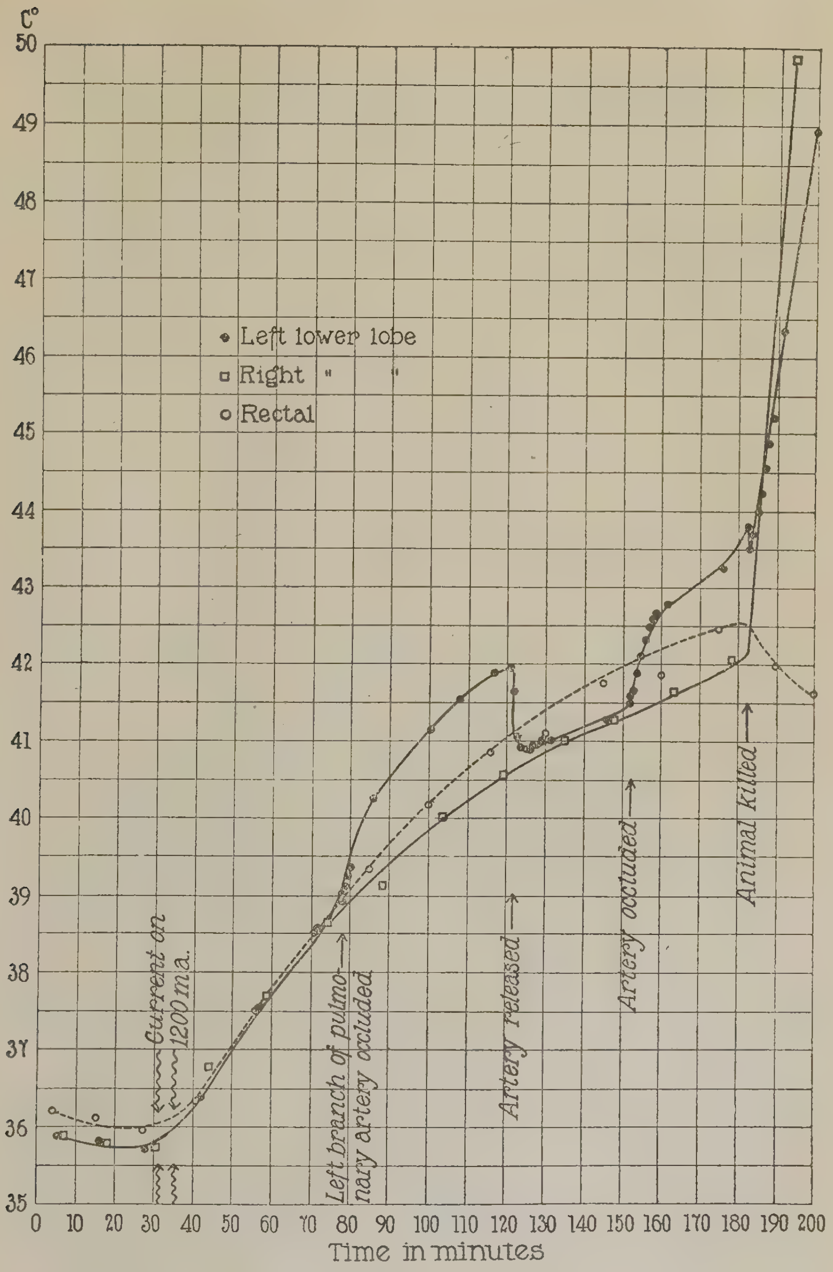

FIG. 4.

Comparison of heat developed in right and left lungs and rectum, with effect of clamping and releasing artery to the left lung.

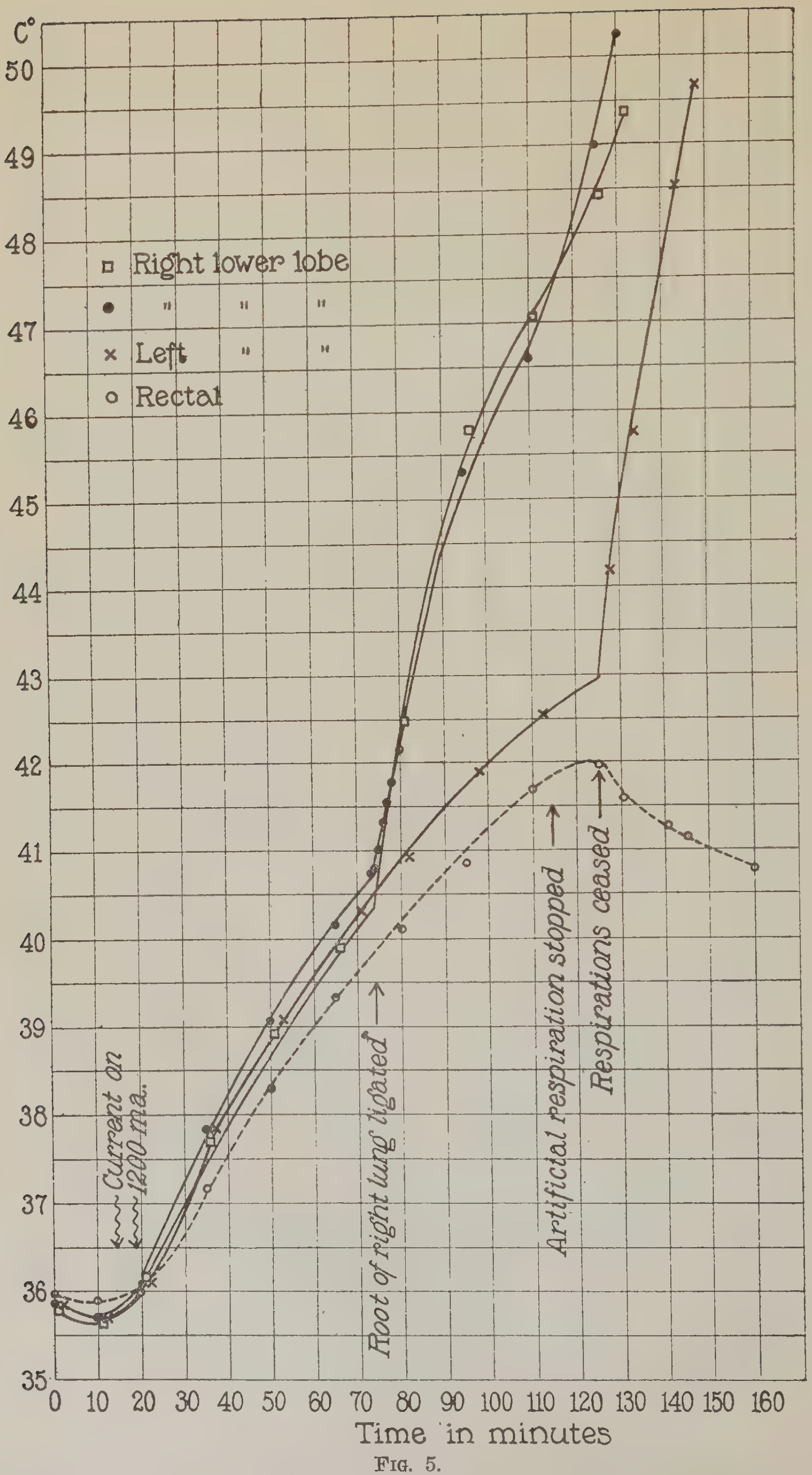

Fig. 5.

Comparison of heat developed in right and left lungs and rectum. Effect of ligating the root of the right lung, and effect of sudden death.

These and other experiments indicate that the efficiency of the local circulation determines whether the heat produced by the passage of high frequency currents through the living animal will remain localized or be disseminated uniformly throughout the body.

This is a preliminary report.

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3517

Observations Upon Certain Isolated Loops in Intestinal Obstruction.

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In previous experiments[1] loops of intestine were doubly occluded and the continuity of the intestinal tract was re-established by anastomosis between the proximal and distal ends. (Fig. 1. a.) At the same time, nothing was done to interfere with the mesenteric circulation of these blind intestinal loops. A fatality occurred if the loop subsequently twisted so as to occlude the mesenteric circulation, or if the loop distended sufficiently to interfere with the circulation of the vasa recta through intra-enteric pressure. If the above factors did not obtain, the animal, in each case, made an uneventful recovery.

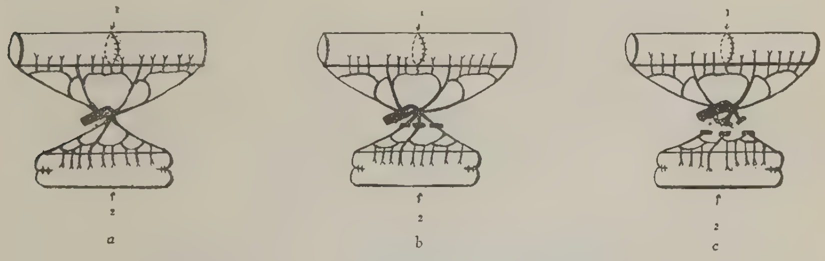

FIG. 1.

a No deliberate interference to mesenteric circulation.
b Mesenteric circulation ligated.
c Mesenteric circulation ligated and cut.
1 Reconstruction of canal.
2 Isolated loop.
Black oblong = deliberate interference to mesenteric circulation.

Additional experiments showed that scraped, washed intestinal mucosa, when introduced into the peritoneal cavity, gave one of these results: the animal made an uneventful recovery, or the animal died as the result of a peritonitis, or the animal developed symptoms and signs of an intoxication similar to that of intestinal obstruction.

Dragstedt et al.[2] showed that sterile devitalized segments of intestine were compatible with life.

The following experiments were divided into four groups:

TABLE I.

Group	Experimental technic	Total animals used†	Average duration of life	Necroscopy and remarks
1*	Immediate occlusion of circulation Loop pedicle intact Fig. 1 b	2	62 hours	Loops slightly distended, dark red discoloration Sero-sanguinous fluid in peritoneal cavity
2*	Gradual occlusion of circulation Loop pedicle intact Fig. 1 b	3	38 hours	Loops distended, bluish-black discoloration Sero-sanguinous fluid in peritoneal cavity
3*	Immediate occlusion of circulation Loop pedicle divided Fig 1 c	3	32 hours 1 animal 2 animals still living	Loops slightly distended, dark red discoloration Sero-sanguinous fluid in peritoneal cavity Two animals still living—symptomless
4*	Gradual occlusion of circulation Loop pedicle divided Fig. 1 c	5	38 hours	Loops slightly distended, dark red discoloration Sero-sanguinous fluid in peritoneal cavity

*Operation common to all experiments. A 20 to 30 cm. intestinal segment was isolated and ends were occluded; continuity of the intestinal canal was reconstructed.

†Variations, 16 animals not included in above table.

These experiments apparently establish the fact that an isolated loop with or without intact pedicle can produce a lethal agent, absorbable through the capillaries or lymphatics of the peritoneum. The result may be fatal without the production of a bacterial peritonitis. The clinical picture and necroscopy findings are similar to those of segmental or loop intestinal obstruction. In some instances the animals made an uneventful recovery, whereas in others a generalized peritonitis resulted.

This is a preliminary report.

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[2] Dragstedt, L. R., Moorehead, J. J., Burcky, F. W., *J. Exp. Med.*, 1917, xxv, 421.

3518

A Simple Respirometer for Microbial Respiration.

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From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

Since the comprehensive work of Novy, Roehm and Soule[1] on microbic respiration appeared in 1925, an effort has been made to devise a simple apparatus for determining CO_2 production in cultures which would be simple and available for ordinary laboratory use. It is doubtful if any device known at present will give the fine results which Novy, Roehm and Soule are able to obtain with the compensation manometer.

Bronfenbrenner[2] described a simple micro-respirometer in 1926 using as a test object a strain of bacteriophage. He was unable to demonstrate that the bacteriophage respired. In a more recent report this author has studied the respiration of the herpes virus and of rabies virus[3]. In this study in order to differentiate between the respiration of tissue and that of the filterable virus he made use of the Warburg[4] respirometer to determine the oxygen uptake and of his closed cell respirometer for the respiration proper.

Bertrand[5] was perhaps the first to make use of an alkali trap for the absorption of respired CO_2. In searching for some simple method to demonstrate microbial respiration to students in bacteriology we decided to make use of this simple procedure. From ordinary culture tubes containing smaller tubes within, which served for a trap, a more elaborate apparatus has been devised by which it is possible to make quantitative tests for respiration as well as qualitative tests.

Figure 1 shows such an apparatus in the form of a flask carrying a side chamber which serves as the trap. The flask E is of 100 cc. capacity. It is entered through stop-cock B and can be exhausted

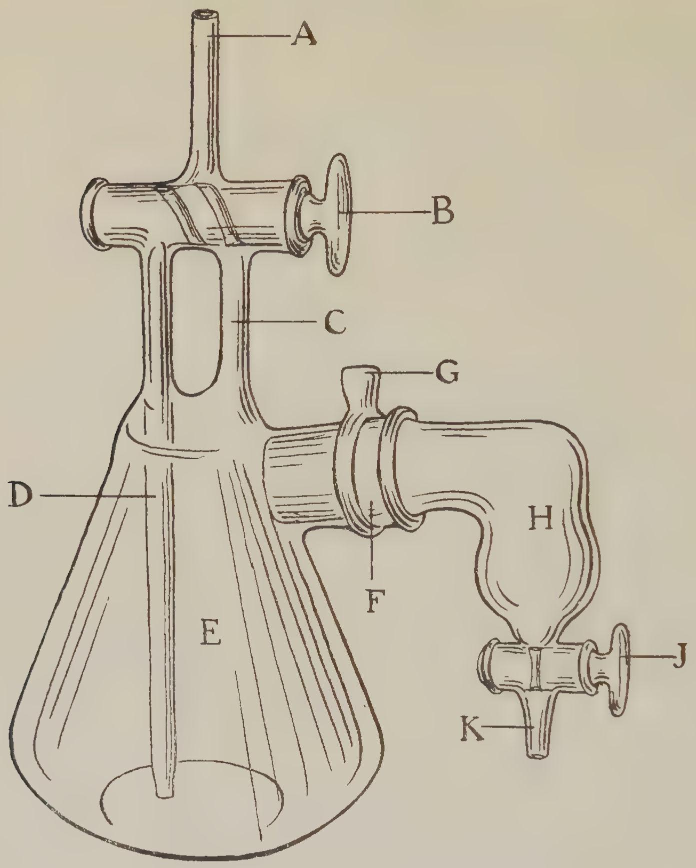

FIG. 1.

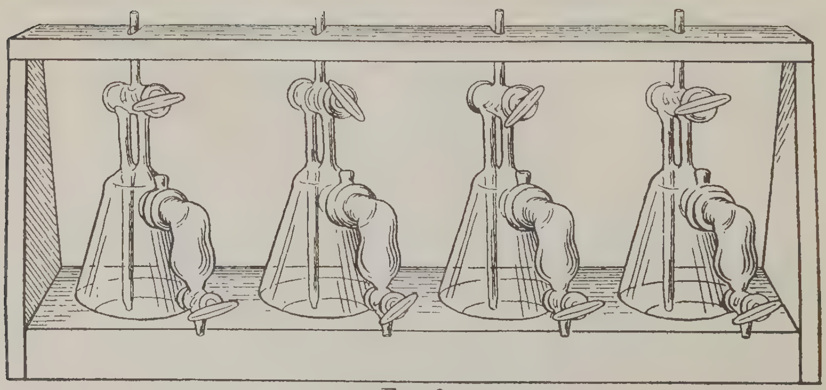

FIG. 2.

through C. Substances can be introduced and withdrawn through inlet tube D. The side chamber H is fitted into flask E through a ground joint F and this joint is further protected by a mercury seal G. The side chamber has an outlet through stopcock J through the nipple K. Figure 2 shows a series of the apparatuses in a special rack for control work.

The broth culture is first placed in flask E (20 cc. of culture material usually being employed) and 5 cc. of distilled water containing an indicator (usually phenol red) and a known quantity of carbonate free alkali is placed in the side chamber H. After a given

TABLE I.

CO_2 Determination in Duplicate					Average 1st series	Average 2nd series	1st series corrected for broth	2nd series corrected for broth
Sample	Vol. per cent CO_2 First Series		Vol. per cent CO_2 Second Series					
Broth	4.4	4.4	4.2	4.0				
Flask 1	14.7	14.6	14.9	14.5	14.65	14.7	10.25	10.6
Flask 2	14.7	14.7	15.0	14.7	14.7	14.85	10.3	10.75
Flask 3	9.8	9.8	8.9	8.8	9.8	8.85	5.4	4.75
Flask 4	10.9	10.7	9.2	9.0	10.8	9.1	6.4	5.0
Trap 1	9.6	9.6	10.2	10.7	9.6	10.4	—	—
Trap 2	9.5	9.6	9.9	10.5	9.55	10.2	—	—
Trap 3	9.2	9.2	8.3	8.0	9.2	8.15	—	—
Trap 4	9.1	9.0	7.9	7.8	9.05	7.85	—	—

period of incubation the contents of E and of H or a mixture of the two can be examined for CO_2 content by the Van Slyke CO_2 apparatus.

A typical protocol in which no additional oxygen is added to the apparatus follows:

CO_2 production was determined upon a culture of Coli "D," which is sensitive to a strain of bacteriophage. An equal amount of a young broth culture of B. coli "D" was added to the 20 cc. of beef extract broth pH 7.6 in flasks E, Figure 2. To flasks one and two .5 cc. of B. coli "D" bacteriophage was also added. In side chamber H, 1, 2, 3 and 4 5 cc. of distilled water containing 5 drops of a .2 per cent phenol red solution and 10 drops of a carbonate free N/20 NaOH, were introduced. F was sealed at G with mercury. The set-up was placed in the incubator for 24 hours and CO_2 determinations were made at the end of that period upon the culture fluid and upon the contents of the alkali trap with the Van Slyke CO_2 apparatus.

It will be noted in Table I that the reading obtained with the uninoculated broth in the Van Slyke apparatus, when corrected, gives 4.4 cc. volume per cent of CO_2. The culture material in apparatus 1 and 2 gave readings consistently higher than the culture material in apparatus 3 and 4. Apparatus 1 and 2 contained bacteriophage, while 3 and 4 did not. Again the determination of CO_2 in the trap showed that the trap in apparatus 1 and 2 contained more CO_2 than did the trap in apparatus 3 and 4. When these readings are corrected for the CO_2 content of uninoculated broth it is found that there is about the same quantity of CO_2 bound by the culture fluids by the alkali in the trap. Much of this CO_2 can be released by introducing a strong acid through tube D, but it is obvious that the method has its limitations.

The excess of CO_2 production in apparatus 1 and 2 which contained bacteriophage is not interpreted to indicate that the bacteriophage respires, as it is more likely due to the stimulus to growth imparted to the sensitive strain of the test organism. Attention has been drawn to this phenomenon elsewhere.[6]

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[1] Novy, F. G., Roehm, H. R., and Soule, M. H., *J. Inf. Dis.*, xxxvi, Nos. 2, 3, 4.

[2] Bronfenbrenner, J. J., *Science*, 1926, lxiii, 51.

[3] Bronfenbrenner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 176.

[4] Warburg, O., *Biochem.* 2., 1923, cxlii, 317.

[5] Bertrand, G., *Annal. de l'Inst. Pasteur*, xxvi, 852-857.

[6] McKinley, E. B., and Holden, M., *J. Inf. Dis.*, xxxviii, No. 6, 451-456.

3519

The Influence of the Vegetative Nerves Upon the Distribution of Arsenic After Salvarsan Injections.

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The present investigation gives a comparison between the distribution of arsenic in normal animals[1] and animals which were under the influence of epinephrin. Epinephrin was given subcutaneously twenty minutes before the salvarsan was injected intravenously. In Table I the figures in column (A) indicate the arsenic content in mg. per 100 gms. dried specimen of the various organs after an intravenous injection of 80 mg. salvarsan per kilo body weight, preceded by the subcutaneous injection of 5 mg. per kilo body weight of epinephrin. The figures under (B) are given for comparison and represent arsenic determinations in rats which received intravenous injections of 160 mg. of salvarsan per kilo of body weight without epinephrin. A dose of 80 mg. of salvarsan is equivalent to 25 mg. of metallic arsenic and a dose of 160 mg. of salvarsan contains 50 mg. of metallic arsenic.

TABLE I.
Average arsenic content of organs.

Interval	Blood		Liver		Spleen		Brain	
	A.	B.	A.	B.	A.	B.	A.	B.
Immediate	71.17	14.76	71.05	9.33	18.39	42.53	1.230	39.12
5 min.	90.96	42.21	96.48	33.84	30.59	32.84	0.911	5.51
10 min.	72.63	15.96	109.04	17.47	27.12	66.49	0.880	3.85
20 min.	77.39	13.72	102.33	96.02	20.36	61.16	0.215	1.90

Table II shows the percentage of the arsenic, actually injected, that is present in the organs at the given intervals. Here again the

TABLE II.
Percentage of arsenic remaining in tissues of rats.

Interval	Blood		Liver		Spleen		Brain	
	A.	B.	A.	B.	A.	B.	A.	B.
Immediate	53.62	5.12	35.52	2.66	3.67	4.25	0.07	1.20
5 min.	68.08	15.80	48.24	8.46	6.12	3.28	0.06	0.18
10 min.	54.36	5.97	54.52	4.37	5.42	6.65	0.05	0.12
20 min.	57.92	5.12	51.12	24.00	4.07	6.12	0.02	0.09

figures in column (A) represent salvarsan preceded by epinephrin and those under (B) salvarsan alone.

The results of these studies can be briefly summarized as follows: Under normal conditions arsenic injected in the form of the disodium salt of salvarsan intravenously into the mammalian body is immediately distributed to the various organs. Only 5.12 per cent of the amount actually injected remains in the blood stream immediately after the injection while 94.88 per cent must either be distributed in the tissues or be excreted. Shortly after this general distribution a reorientation of all organs sets in. As demonstrated in former work,[2] there is an increase in the action of the liver and other abdominal organs accompanied by a dilation of their vessels. At the same time, the activities as well as the vascular capacity of the peripheral organs are diminished. Simultaneously with this typical rearrangement of almost all organs, arsenic disappears from the peripheral organs, of which the brain represents one part. Arsenic reappears in the blood stream and is gradually accumulated in the liver with a corresponding decrease of the arsenic findings in blood and other organs. Fig. 1 shows the arsenic content in mg. per 100 gm. dry specimen in the organs at different intervals after the injection of salvarsan alone and salvarsan preceded by epinephrin.

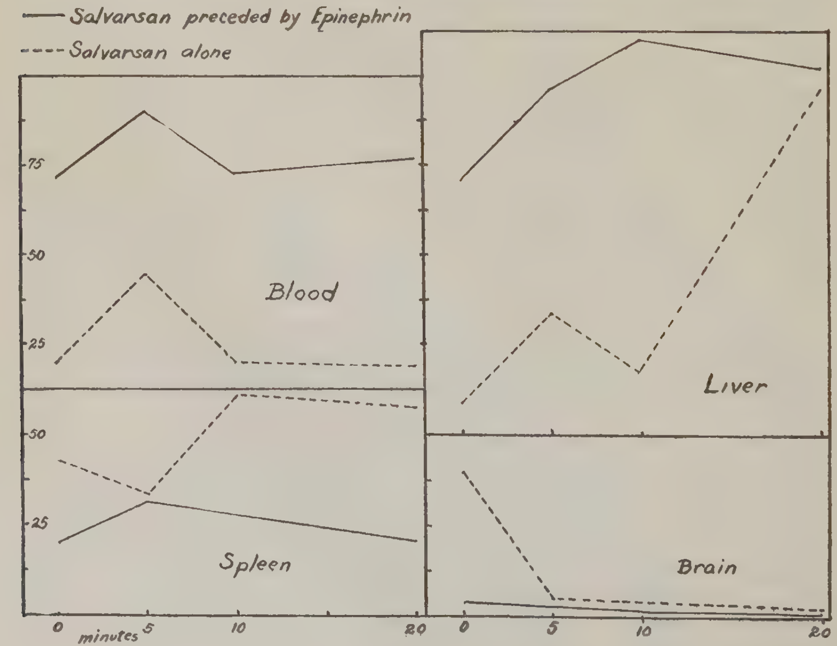

FIG. 1.

If epinephrine is given previous to the injection of salvarsan the distribution of arsenic is markedly changed as far as the actual values are concerned. Instead of 5 per cent of the amount of arsenic actually injected 53.62 per cent remains in the circulation immediately after the injection, so that only 46.38 per cent (minus the excreted portion) can be distributed into the organs. These figures parallel the arsenic content of the brain which is 39.12 mg. per 100 gms. dry specimen in normal rats and only 1.22 mg. in epinephrinized animals. The percentage of the total dose of arsenic actually injected which finds its way to the brain is 1.2 per cent under normal conditions as compared with 0.07 per cent in epinephrinized animals.

The discussion of the individual figures and their significance will be left to a more detailed publication. However, it may be stated that, if the involuntary nervous system is under the influence of epinephrin, almost five times as much arsenic is retained in the blood stream and only a small percentage of that normally distributed into the brain reaches that organ. It is also evident that the second phase of the arsenic distribution, namely the reappearance of arsenic in the blood and its gradual accumulation in the liver, is similarly altered. However, it is interesting to note that even although the percentages are much lower, for both phases of the arsenic distribution in the epinephrinized animals, still they are very similar to those in normal rats. The remarkably low figures for the arsenic in the brain show how much its penetration into the tissues is inhibited as compared with the normal control animals.

Studies with arsenic distribution in rats under the influence of epinephrin show how essential a normal state and a normal undisturbed activity of the vegetative nerves are for the distribution of arsenic, therapeutically administered, in a mammalian body.

This is a preliminary report.

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[1] Fordyce, John A., Rosen, I., and Myers, C. N., *Am. J. Syph.*, 1924, viii, 377.

[2] Müller, E. F., *Munch. med. Woch.*, 1926, lxxiii, 9.

Müller, E. F., Myers, C. N., and Metz, G. P., *Arch. of Derm. and Syph.*, 1927, xv, 186.

3520

Influence of Organic Salts on the Action of Tyrosinase

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With the object of finding an analogy to the inhibition phenomena observed by us in precipitin reactions with azoproteins[1] we examined the effect of the sodium salts of organic acids on the action of the oxidase of potatoes and mushrooms on tyrosin. Many were found to have an inhibiting effect upon the ferment action, but the

TABLE I.

0.1 cc. of potato extract (1 gram of potato was extracted with 10 cc. of water and the solution was filtered) was added to a mixture of 0.4 cc. of a solution of tyrosin (10 milligrams in 10 cc. water) and 0.05 cc. of the solutions containing 1 millimole of the sodium salt of the acid in 10 cc. of water. The tests were kept in the room for 2½ hours. To determine the intensity of color, the tests were compared with a series of dilutions made from a solution equal in color to the control tube containing tyrosine and ferment only.

Substances used: 1 acetic acid, 2 propionic acid, 3 butyric acid, 4 valeric acid, 5 heptylic acid, 6 chloracetic acid, 7 trichloracetic acid, 8 brombutyric acid, 9 cyanacetic acid, 10 tartaric acid, 11 citric acid, 12 acrylic acid, 13 crotonic acid, 14 fumaric acid, 15 ethylsulphonic acid, 16 benzolsulphonic acid, 17 cyclohexane carboxylic acid, 18 benzoic acid, 19 α naphtoic acid, 20 β naphtoic acid, 21 furancarboxylic acid, 22 thiophene carboxylic acid, 23 picolinic acid, 24 phenylactic acid, 25 cinnamic acid, 26 hydrocinnamic acid, 27 orthomethylbenzoic acid, 28 metamethylbenzoic acid, 29 paramethylbenzoic acid, 30 orthohydroxybenzoic acid, 31 metahydroxybenzoic acid, 32 parahydroxybenzoic acid, 33 orthochlorbenzoic acid, 34 metachlorbenzoic acid, 35 parachlorbenzoic acid, 36 orthobrombenzoic acid, 37 metabrombenzoic acid, 38 parabrombenzoic acid, 39 orthonitrobenzoic acid, 40 metanitrobenzoic acid, 41 paranitrobenzoic acid.

Numbers of the substances	Control	1	2	3	4	5	6	7	8	9
Intensity of color	10	9½	8½	9	8½	8½	9½	9	1½	10

Numbers of the substances	10	11	12	13	14	15	16	17	18	19
Intensity of color	9½	9	9	9	9	9	10	4½	0	1½

Numbers of the substances	20	21	22	23	24	25	26	27	28	29
Intensity of color	0	8	1½	0	8½	2½	8	5	0	0

Numbers of the substances	30	31	32	33	34	35	36	37	38	39
Intensity of color	½	0	½	4	0	0	7	0	0	9

Numbers of the substances	40	41
Intensity of color	3	0

degree of inhibition varied greatly with the different acids. In a general way it can be stated that the carboxylic acids of benzene and of other cyclic compounds acted more strongly than fatty acids, and that stronger inhibition was caused by the meta and para substituted acids than by the ortho substituted. The tests were made with solutions neutral to litmus, and also in the presence of buffers. Most of the substances acted similarly on mushroom extract and on potato extract, but in some instances differences were observed.

A part of the results is presented in Table I, the figures indicating the degree of oxidation as judged from the intensity of the color.

Somewhat analogous phenomena were noticed when in place of ferment, iron salts and H_2O_2 were used for the oxidation of tyrosin.

A similar difference in the action of ortho, meta and para substituted aromatic acids was also seen in their inhibiting effect upon the color reaction of sodiumsalicylate with ferric chloride. Apparently this was due to the formation of iron compounds which separated in the form of precipitates, but the effect was the same when the precipitation was prevented by addition of a solution of gelatin.

This is a preliminary report.

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[1] Landsteiner, K., *Biochem. Z.*, 1920, civ, 280.

3521

Further Studies on the Heterogenetic Haptene.

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In continuation of the studies recorded previously[1] the following observations were made.

The crude material obtained from horse kidney by extraction with alcohol, and freed from ether soluble substances in the manner described previously, was extracted with boiling benzene. From the solution on standing a precipitate separated out which was removed by centrifugalization. The solution was concentrated. On addition of an equal volume of alcohol a precipitate formed. This was dissolved in benzene and precipitated with alcohol, and the procedure was repeated many times until there appeared in the benzene solution a substance giving a distinct copper orcinol reaction. The final precipitate was dissolved in water and precipitated from the solution with two volumes alcohol, and was then extracted thoroughly with methyl alcohol and with chloroform. The fraction soluble in methyl alcohol (A) was water soluble and gave a strong orcinol copper test. The insoluble part (B) did not give this reaction and was practically insoluble in organic solvents. With hot water it yielded a viscous pseudosolution. This solution gave a flocculent precipitate on addition of Fehling's reagent. On hydrolysis of "B" with 3 per cent H_2SO_4, after about 30 minutes, an insoluble substance settled out and the solution reduced Fehling's reagent and gave a crystallized osazone. After hydrolysis for 12 hours the quantity of sugar amounted to about 35 per cent, calculated as glucose. After precipitation with Fehling's solution substance B contained neither P nor S. The material was active in the complement fixation test with heterogenetic immune serum and produced heterogenetic hemolysins in rabbits when several injections of 1 mg. each of the substance mixed with pig serum were given. Even quantities as small as 0.2 mg. had still noticeably immunizing properties. Fraction "A" was distinctly less active in the immunization experiment, though similar in activity to "B" when tested *in vitro*. It should be mentioned, however, that it is apparently difficult to prepare suitable solutions of substance "B".

In a simpler way a product apparently identical to "B" could be prepared by treating the crude material, mentioned in the beginning, with hot pyridine. The crude material is dissolved in 20 parts of hot pyridine and allowed to stand in the refrigerator for 24 hours. A voluminous sediment is then formed. The filtrate contains the principal part of the substance similar to "B."

For the preparation of substance "B" the filtrate is concentrated under reduced pressure to nearly dryness. The residue is taken up in hot methyl alcohol and poured into acetone. A precipitate is formed. This is removed by filtration and dried. The dry material is extracted with 10 parts of a mixture of equal parts of chloroform and of methyl alcohol. The insoluble part is again treated in the same manner. The residue is dissolved in 100 parts of hot water. The solution is allowed to cool and half the volume of Fehling's solution is added. A flocculent precipitate is formed. It is washed with a small volume of water. It is then suspended in water and acidulated with hydrochloric acid. To the opalescent solution alcohol and ether, or acetone is added to precipitate the material. The individual samples of the substance still varied in their elementary composition. The yield of substance "B" was about 1.5 gr. per 100 gr. of crude material when 2 extractions with pyridine were made.

From the pyridineinsoluble material a water soluble fraction similar to "A" was prepared, giving strong orcinol copper reaction and only weak complement fixation with heterogenetic antiserum. Substances of similar chemical properties as "A" could be obtained from beef kidney and beef brain.

This is a preliminary report.

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[1] PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 343-344; *J. Immunol.*, 1925, x, 731-733.

3522

Mononuclear Cells of Blood in Relation to Clinical Findings in Human Tuberculosis.*

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In 1925 a new hypothesis in regard to the propagation and resistance of tuberculosis was presented by Cunningham, Sabin, Sugiyama and Kindwall.[1] The data for their conclusions were obtained from a study of rabbits injected with viable bovine tubercle bacilli; differential blood studies were made throughout the course of the infection by the supra-vital technique of Sabin, and freshly autopsied material was examined by the same technique. They found that, during the course of a tubercular infection, the so-called "reticular cells" are stimulated to an increased production of monocytes and these in turn are transformed into typical epitheloid and giant cells. From a study of the appearance and number of the bacilli, which were found in the monocytes and in the epitheloid cells, they concluded that these cells phagocytize organisms but are unable to destroy them, and hence act virtually as culture tubes. The concept was thus advanced that the monocytes are stimulated, in tubercular infections in rabbits, to such an increase in number and in phagocytic capacity, that, inasmuch as they seem unable to destroy the bacilli, they aid in increasing and disseminating the infection. Furthermore, the authors showed that these changes became more marked as the disease progressed, and receded with improvement; and that, coincident with the numerical changes in the monocytes, the lymphocytes were effected in inverse ratio.

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* This work has been assisted by a grant from the Research Committee of the National Tuberculosis Association.

The same technique is now being employed in similar studies upon clinical cases of human tuberculosis. All of the cases so far studied have been patients in the Vanderbilt University Hospital, selected for study, irrespective of the site or grade of infection or age of the patient. Where cases are in the hospital sufficiently long, or where marked clinical changes occur, the progress is followed by repeated examinations; and whenever tubercular fluids are obtained for clinical reasons they are subjected to study by the supra-vital technique, as are also tissues secured by autopsy or biopsy. Furthermore, we are extending our study to such other types of disease entities as show any indications of changes in the monocytes or lymphocytes. And finally, intracutaneous tuberculin tests are made in conjunction with these studies.

The blood and tuberculin findings thus obtained are being analyzed against the various clinical factors such as history, duration, physical examination, progress, X-ray plates, laboratory findings, biopsy and autopsy reports and the result of animal inoculation.

We have studied about 50 cases up to the present time in which tuberculosis has been positively diagnosed or strongly suspected. From the analyses of these 50 human cases it has been found that cytological changes, similar to those reported in the rabbit with experimental bovine tuberculosis, occur in about 85 per cent of them. Those cases showing numerical and qualitative changes in monocytes and lymphocytes, comparable to the changes described in the rabbit, are usually those with pulmonary or disseminated tuberculosis; while only a smaller percentage of the more localized types of tuberculosis present characteristic blood changes. Moreover, the same variations, as those occurring in the rabbit during the course of experimental tuberculosis, have been found in the human cases in which the disease progress has been followed; *i. e.*, the numbers of monocytes rise and of lymphocytes fall as the disease progresses and the opposite changes occur when resistance to the infection develops.

As the outcome of these changes in numbers of monocytes and lymphocytes, it is found that the ratio between them increases in proportion with the severity of the invasion and decreases in proportion to improving resistance. In general, this ratio is found to be a very good index of the condition of the patient. We have called this value the "monocyte-lymphocyte ratio."

This is a preliminary report.

While the findings thus enumerated correspond to those reported in the rabbit with generalized tuberculosis, the more chronic type of the disease in the human, and the greater tendency to differences in localization and to the complication by other disease factors, makes the findings in the human more difficult to analysis, and hence requires a greater number of studies before conclusions may be drawn.

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[1] Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Johns Hopkins Hosp. Bull.*, 1925, xxvii, 231-280.

3523

Oral Immunization of Rats Against Pneumococcus Types 2 and 3.

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We have previously reported on the successful immunization of white rats against pneumococcus Type 1 by oral administration of the living[1] or acid killed germ.[2] We wished to see whether our results with Type 1 could be duplicated with Types 2 and 3. The organisms were grown on glucose meat extract media but no HCl acid was added to kill the growth. After centrifuging, the germs were mixed with cracker meal and water, and growth from 50 ccm. was fed to each rat daily for 18 to 21 days. Tests were made by injecting intraperitoneally in volume of 0.20 ccm.

When the first feeding experiment with Type 2 was completed it was found that the treated animals tolerated large doses, but that the controls did the same. On reexamining virulence tests which had been made earlier, and, on extending these tests, it was learned that small untreated rats (up to ca. 85 to 95 grammes) were relatively as susceptible (with exceptions) to Type 2 as to Type 1, 10^{-7} and 10^{-6} cc. being fatal doses. However, rats, from this range of weight and above, showed a distinct tendency to rapidly increasing resistance. The age or weight at which this relative immunity begins to develop cannot be given more definitely, since individuals in the same group as well as whole groups of animals vary in this respect. The increased resistance is entirely out of proportion to the increased weight, and at its maximum is of the magnitude obtained to Type 1 when this germ is fed. It may be that the tolerance of the larger animals to Type 2 follows, with changing diet, the ap-

TABLE I.

Immunizing effect of feeding Type 2 to small rats before they have developed a natural partial immunity. S = Survived. D = Died-days. C = Control. E = Experimental.

Date	Weight	Dose	Result	Date	Weight	Dose	Result	Date	Weight	Dose	Result
	gm.	cc.			gm.	cc.			gm.	cc.	
Jan. 7	C 53	10^{-6}	D2	Jan. 22, 1927	C 72	10^{-4}	D3	Jan. 27	C 112	10^{-3}	D2
	C 52	10^{-7}	S		C 71	10^{-5}	S		C 114	10^{-3}	D1
	E 53	10^{-6}	D2		C 71	10^{-5}	D2		E 108	10^{-3}	S
	E 53	10^{-7}	D2		C 66	10^{-6}	S		E 104	10^{-3}	S
					E 78	2×10^{-3}	S				
Jan. 19	C 76	10^{-6}	S		E 73	10^{-4}	S	Jan. 29	C 108	10^{-3}	D1
	C 67	10^{-7}	S		E 72	10^{-5}	S		C 92	10^{-3}	D2
	E 68	10^{-6}	S		E 68	10^{-6}	S		E 96	10^{-3}	D1
	E 64	10^{-7}	S						E 108	10^{-3}	S
				Jan. 25, 1927	C 85	2×10^{-3}	D1				
Jan. 21, 1927.	C 87	10^{-4}	D2		C 84	10^{-4}	S	Jan. 31	C 104	10^{-2}	D1
	C 87	10^{-5}	D2		C 85	10^{-4}	S		C 104	10^{-3}	D1
	C 77	10^{-6}	D2		C 82	10^{-5}	S		C 109	10^{-3}	*
	C 76	10^{-7}	S		C 82	10^{-6}	S		E 109	10^{-2}	S
	E 87	10^{-4}	D2		E 92	2×10^{-3}	S		E 107	10^{-3}	S
	E 87	10^{-5}	S		E 91	10^{-4}	D2		E 105	10^{-3}	D3
	E 83	10^{-6}	S		E 85	10^{-4}	S				
	E 77	10^{-7}	S		E 84	10^{-5}	D2				
					E 83	10^{-6}	S				

* Comatose; killed.

TABLE II.

Immunizing effect of feeding rats Type 3. S = Survived. D = Died-days. E = Experimental. C = Control.

Date	Weight	Dose	Result	Date	Weight	Dose	Result	Date	Weight	Dose	Result
	gm.	cc.			gm.	cc.			gm.	cc.	
Jan. 4	C 112	10^{-6}	S	Jan. 7	C 123	10^{-6}	S	Jan. 21, 1927	C 144	10^{-4}	D2
	C 113	10^{-6}	D2		C 123	10^{-5}	D2		C 132	10^{-5}	D2
	E 104	10^{-6}	S		C 121	10^{-4}	D2		C 130	10^{-6}	D2
	E 109	10^{-6}	S		E 124	10^{-5}	S		C 125	10^{-7}	S
					E 126	10^{-4}	D2		E 150	2.10^{-3}	S
Jan. 5	C 107	10^{-5}	D2		E 129	10^{-3}	D2		E 149	10^{-4}	S
	C 109	10^{-5}	S						E 147	10^{-5}	S
	E 107	10^{-5}	S	Jan. 17	C 127	10^{-5}	D2		E 136	10^{-6}	S
	E 112	10^{-5}	S		C 136	10^{-5}	D2				
					E 125	10^{-5}	S	Jan. 22, 1927	C 152	10^{-4}	S
Jan. 6, 1927	C 117	10^{-6}	D2		E 126	10^{-5}	S		C 148	10^{-5}	D2
	C 118	10^{-5}	D2						C 142	10^{-6}	S
	C 121	10^{-4}	D2	Jan. 19, 1927	C 137	10^{-4}	D2		C 142	10^{-7}	S
	E 121	10^{-6}	S		C 136	10^{-5}	D2		E 174	2.10^{-3}	D2
	E 123	10^{-5}	S		C 134	10^{-6}	D2		E 183	2.10^{-3}	D2
	E 127	10^{-4}	S		C 134	10^{-7}	S		E 173	10^{-4}	S
					E 135	10^{-4}	S		E 168	10^{-5}	S
					E 149	10^{-5}	S				
					E 139	10^{-5}	S				
					E 126	10^{-6}	S				

pearance among the intestinal flora of an organism closely related immunologically to this germ.

The results of the first feeding experiment, before it was known that the *larger* animals develop a natural immunity, show that feeding of Type 2 to such rats does not produce an additive effect. The Type 2 experiment was repeated using small rats in the hope that they could be treated and tested with controls before either group developed the normal resistance exhibited by larger animals. Table 1 gives the data. On January 7 no immunity had yet been established. On the 19th both controls and treated rats survived 10^{-7} and 10^{-6} cc., illustrating an increased resistance of untreated rats referred to above. On the 21st 10^{-6} cc. and 10^{-5} cc. killed controls, but not experimental rats. On the 22nd 2×10^{-3}, 10^{-4} and 10^{-5} cc. failed to kill treated rats whereas the controls succumbed. On the 25th the results are somewhat irregular, the natural immunity of the growing rat is again evident. On the 27th, 29th and 31st the increased resistance of the treated animal is demonstrated. The protective effect of feeding is apparent, although more difficult to show than for Type 1.

In Table 2 are given the results obtained with feeding of Type 3. Untreated large rats are not less susceptible to Type 3 than young rats, hence the demonstration of the immunity produced by feeding is not encumbered by the difficulty experienced with Type 2. The data show clearly the protection afforded by feeding Type 3.

We have not yet attempted feeding Group 4. However, preliminary virulence tests with a member of this group indicate that, just as for Type 2, the larger animals normally exhibit an immunity not possessed by young ones.

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[1] Ross, Victor, *J. Immunol.*, 1926, xii, 237-249.

[2] Ross, Victor, *J. Lab. and Clin. Med.*, 1927, xii, 566-571.

Peking Branch.

Peking Union Medical College, March 10, 1927.

3524

The Effect of Instillations of Ephedrine Solution Upon the Eye.

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The sudden and great popularity of ephedrine for therapeutic purposes, chiefly in connection with mucous membrane affections, seemed to us to warrant further investigations on the action of the drug upon the eye. Experimental tests with ephedrine hydrochloride ($C_{10}H_{15}ON. HCl$) in solutions ranging from 1 to 10 per cent, were made on the eyes of 15 individuals. It was our object to determine what effect, if any, the drug has upon the acuity of vision, the sensitivity of the ocular mucous membrane, the intraocular tension, the pupil, the range of accommodation, and in producing any other symptoms, subjective or objective. Two drops of the solution were instilled 4 times into each eye at intervals of 5 minutes. Observations were made continuously over periods of several hours.

Results—1. Vision: There was no diminution of vision, but all the subjects noticed a slight fading of the test letters, which cannot be attributed to a loss of rectinal sensitivity, but rather to a lack of definition of the letters due to the enlarged pupil.

2. Anesthesia or hyperesthesia: No diminished or increased sensitivity of the cornea and conjunctiva was observed.

3. Pupils: Mydriasis occurred in all subjects. The first evidence of dilatation of the pupil was observed as early as 7 minutes after the first instillation in one case. The mydriatic action in another case did not begin until 67 minutes. Generally the first sign was observed in 15 or 20 minutes. Maximum dilatation (about 7 mm.) was attained in the great majority of subjects in 40 to 60 minutes after the first dilatation. In one case there was only moderate mydriasis. The mydriasis lasted from 3 to 5 hours, depending somewhat, but not wholly, upon the strength of the solutions used.

A distinct difference was observed between subjects with light irides and those with dark. In the subjects with light irides (all Caucasians), the mydriatic action of the drug was much more prompt than in those with dark irides (all Chinese). Furthermore, in the former the dilatation of the pupil was wider, and the weaker solutions were more effective. But the mydriasis seemed to disappear more rapidly in those subjects in which it developed more quickly. Roughly speaking, a 20 per cent solution was as effective in producing mydriasis in the light-eyed cases as a 5 per cent solution was in the dark-eyed. Apparently, there is more rapid absorption as well as quicker elimination of the drug in light-eyed persons. That this is due to an obstructive action on the part of the greater number of pigment cells in the conjunctiva and sclera in the region of the limbus, or of the chromatophores in the iris in dark-eyed persons, is only a surmise. There may be a racial difference in the rate of absorption, but that is unlikely, apart from the variation in the number of pigmented cells.

The pupils were active to light throughout all the experiments.

4. Range of Accommodation: All subjects showed a little loss of accommodation, the amount ranging from 0.25 to 2.5 diopters. The amount depended somewhat on the strength of the solution, but not wholly, for in some of the subjects on whom the stronger solutions were used, the greatest cyclopegic effect did not take place. In brief, there appeared to be an individual difference in the action of the drug upon the ciliary muscle, a difference that could not be explained by the character of the refractive error. The onset of the cyclopegia always developed a little later than the onset of the mydriasis, but generally disappeared some time before the mydriatic effect wore off.

5. Intraocular Tension: A Schiötz tenometer, which measures the tension in millimeters of mercury, was used. A solution of holocaine was used to anesthetize the cornea of both eyes, but the ephedrine solution was instilled into one eye only, the other eye being used as a control. No changes in the tension were observed during a period of more than two hours.

6. Other Symptoms: a. Subjective: All the subjects complained of a stinging and burning sensation after the instillations, the degree depending upon the strength of the solutions. The last drop caused about as much discomfort as did the first, confirming the direct test that no anesthesia developed. Several noticed a sticky, stiff feeling in the lids. Those upon whom the stronger solutions were used complained of dull frontal headache which came on towards the end of the first hour, and lasted for an hour or two; two of these sub-

jects had a little nausea. But in those subjects upon whom the weaker solutions were used, the discomfort was only mild, certainly not more than, or even as much as an individual would have following the use of such drugs as holocaine, homatropine, or zinc sulphate.

b. Objective: All the subjects developed a mild congestion of the superficial vessels of the palpebral and bulbar conjunctiva, which lasted for about 20 or 30 minutes. There also developed a pericorneal or ciliary injection, which was quite marked in two subjects which had strong solutions instilled. The appearance of the conjunctiva became normal generally in about half an hour, but was not followed by blanching, as occurs with adrenalin. The corneal epithelium was examined with a corneal microscope and a slit lamp, but no changes, such as occur following the use of cocaine, were observed. Neither was there any protosis of the eyeball, nor a widening of the palpebral fissure, as occur with cocaine.

Summary—1. Ephedrine hydrochloride, in solutions from 1 to 10 per cent, is a mydriatic, quite uniform in action. The mydriasis lasts from three to five hours.

2. Ephedrine is more effective as a mydriatic in individuals with light irides than in those with dark. Weak solutions of the drug produce approximately the same effect on light eyes, as solutions in about double the strength produce on dark eyes.

3. It has a slight or a moderate cycloplegic effect, which passes off in an hour or two.

4. Ephedrine hydrochloride, when instilled into the eye, is somewhat similar in its physiologic action to atropine, homatropine and cocaine respectively, but its action is more like euphthalmine hydrochloride, although not as prolonged as the latter.

5. It seems to be the best drug that is available for use in making ophthalmoscopic examinations, since its mydriatic and slight cycloplegic effects wear off so quickly.

6. It is somewhat toxic when instilled into the eye in solutions stronger than 5 per cent.

7. A solution of 20 per cent ephedrine hydrochloride is recommended for individuals with light irides, and a 4 per cent solution for those with dark irides.

8. These experiments, on the whole, confirm the work of Miura,[1] and of Chen and Schmidt,[2] but are somewhat more comprehensive and specific in their findings.

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[1] Miura, K., *Berlin Klin. Wockenschr.*, 1887, xxxviii, 707.

[2] Chen, K. K., and Schmidt, C. F., *J. Pharm. and Exp. Therap.*, 1924, xxiv, 339.

3525

Blood Changes Following Injections of Typhoid-Paratyphoid Vaccine.

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From the Department of Ophthalmology and Medicine, Peking Union Medical College.

In a study of the reactions produced in non-febrile patients by intravenous injections of typhoid-paratyphoid vaccine (given therapeutically for ocular inflammations[1]) we have verified certain findings reported by other workers, and made other observations not hitherto reported. Food was not eaten immediately before and during the period of the investigations, but water was taken freely in uniform amounts every hour. The patients were given from 3 to 11 vaccine injections, on an average of one dose every second or third day. The dosage was so calculated as to produce approximately the same temperature reactions throughout the treatment. This was generally accomplished by doubling the size of the preceding injection. The fever produced by the first dose lasted on an average for about 36 hours; the fever following the second dose generally disappeared in 18 hours; the fever period for subsequent doses covered about 10 or 12 hours.

About 30 minutes after the injection the patient developed a chill and a leucopenia. In several cases the leucopenia dropped as low as 1500, and was characterized by a fall chiefly in the polymorphonuclears. About two hours later the leucocyte count had returned to normal and then began a leucocytosis (mainly polymorphonuclear), in some cases rising as high as 35,000. The fever usually coincided with the leucocytosis and reached its maximum in five to eight hours after the injection, and then gradually fell by lysis.

During the stage of the chill and leucopenia there was a perceptible fall in the systolic blood pressure, and an approximately proportionate rise in the diastolic pressure. During the stage of the fever and leucocytosis the original systolic pressure was quite uniformly maintained, but there occurred a marked drop in the diastolic pressure, its low point coinciding with the high point of the fever and leucocytosis.

We also tested the antithrombic power of the patients' plasma, and uniformly found the same marked increase in antithrombin as was observed[2] in cases of typhoid fever. This increase began with the chill and was maintained during the rising phase of the fever, but disappeared quickly as the temperature began to fall. This phenomenon was accompanied by a prolongation of the bleeding time.

Studies on the viscosity of the blood also were made in the hope of discovering the factors responsible for the leucopenia and the leucocytosis, but no constant variations were observed.

This is a preliminary report.

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[1] Howard, H. J., *China Med. J.*, 1927, xli, in press.

[2] Mills, C. A., and Kitzmiller, K. V., *Arch. Int. Med.*, 1926, xxxviii, 544.

3526

Is Uricase Present in Soy Beans?

SCHMORL M. LING. (Introduced by F. R. Dieuaide.)

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Némec (1920) reported that a very "active" enzyme, uricase, is present in soy beans, and formulated an oxidative process of decomposing uric acid with the formation of urea. Horvath[2] (1926) remarks that this process may be a possible source of error in the determination of urea nitrogen in blood by the aeration urease method. Since this method has gained wide popularity, it is important to know whether uricase is present or absent in soy beans. Takeuchi and various other workers have reported that the aqueous extract of soy bean had no action on uric acid and many other protein derivatives.

Experiments were arranged under conditions exactly the same as those described by Némec.[1] Potassium urate was dissolved in boiling water (0.3 gm. in 100 cc.). Chinese yellow soy beans were ground to a fine powder, 5 grams of which were poured into an Erlenmeyer flask. Then 100 cc. of the potassium urate solution were added, followed by 5 cc. of toluol. The flask was fitted with a rubber stopper carrying a 3 inch tube and a bubbling tube reaching nearly to the bottom of the flask. The air, after being filtered through glass wool and 10 per cent sulphuric acid (Némec used sublimate) was led into the flask at a rate of 25 liters per 24 hours. In the control flask, 100 cc. of distilled water were used in lieu of potassium urate solution. The ammonia formed was received in a similar flask containing N/50 sulphuric acid. The temperature was kept constant at 35° C. Four experiments gave concordant results. Table I records the results of one typical experiment together with those of Némec.

TABLE I.

Duration	Némec					Author			
	Control		Potassium urate			Control		Potass'm urate	
	H_2SO_4*	NH_3	H_2SO_4*	NH_3	NH_3 from urate	H_2SO_4	NH_3	H_2SO_4	NH_3
hours	cc.	mg.	cc.	mg.	mg.	cc.	mg.	cc.	mg.
24	8.7	5.31	23.4	14.30	8.99	0.4	0.14	0.5	0.17
72			30.6	18.69		0.7	0.24	0.7	0.24
120	11.8	7.21	31.8	19.43	12.22	0.6	0.20	0.7	0.24
144	11.3	6.90	34.2	20.89	13.99	0.6	0.20	0.8	0.27

* 1 cc. of acid equals 0.000611 grams NH_3.

These results show no evidence of the presence of any appreciable amount of uricase in soy beans.

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[1] Némec, A., *Biochem. Z.*, 1920, cxii, 286.

[2] Horvath, A. A., *J. Biol. Chem.*, 1926, lxviii, 343.

3527

Denaturation of Hemoglobin.

HSIEN WU AND KUO-HAO LIN.

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The present study arose from an inquiry into the question: Is the denaturation of hemoglobin reversible? According to general experience denaturation of proteins is irreversible, but reversion of denatured hemoglobin has been reported by Anson and Mirsky.[1, 2] These workers found that when an alkaline solution of hemochromogen was neutralized, a small amount of hemoglobin was obtained. On the basis of this observation they concluded that there is a reversible equilibrium between hemochromogen and hemoglobin:

$$\text{Hemoglobin} \rightleftarrows \text{Hemochromogen},$$

and that the position of the equilibrium depends on hydrogen ion concentration. Adding acid or alkali to hemoglobin converts it into hemochromogen, while neutralizing hemochromogen turns it into hemoglobin.

The term hemochromogen is used by these authors to designate, not the reduced iron pyrrol complex which they called hem, but a compound of this substance with globin. Since the solubility of hemochromogen is similar to that of denatured proteins, and since it is formed under conditions which would cause denaturation of proteins, they considered hemochromogen as denatured hemoglobin.

The work of Anson and Mirsky was entirely qualitative, and they stated that the evidence for hemochromogen equilibrium was not conclusive.

We denatured different forms of hemoglobin with acid, alkali, heat and alcohol. We allowed the products so obtained to revert in faintly alkaline solution in which they were soluble. Our work definitely establishes the reversibility of the hemoglobin-hemochromogen equilibrium, but our interpretation differs from that of Anson and Mirsky.

Our findings are as follows:

1. Reduced hemoglobin when treated for a short time with dilute HCl is completely precipitated on neutralization. The precipitate, when dissolved in faintly alkaline solution, reverts to hemoglobin. Under suitable conditions the reversion is practically quantitative. If the acid-reduced hemoglobin solution is exposed to the air before it is allowed to revert, no reversion is obtained. If the exposed solution is reduced with hydrosulphite, reversion can again occur.

2. Reduced hemoglobin subjected to the action of alkali, alcohol or heat also shows reversion, but it is much less complete than in the case of acid treated solution. The behavior of the "denatured" hemoglobin towards air in these cases is the same as in acid solution.

3. Methemoglobin behaves towards acids just like reduced hemoglobin. Methemoglobin "denatured" by alkali, alcohol or heat shows no reversion. But if the "denatured" methemoglobin is reduced by hydrosulphite, some reversion is obtained.

4. Oxyhemoglobin "denatured" by acid shows partial reversion. The hemoglobin formed by reversion in this case is methemoglobin.

5. Oxyhemoglobin is only partially converted into methemoglobin by the action of acid. This explains the finding of Roaf and Smart[3] and of Quagliariello[4] that acid liberates from oxyhemoglobin only half as much oxygen as does ferricyanide, and brings it into harmony with the conclusion reached by Conant[5] from electrochemical studies, that methemoglobin bears the same relation to hemoglobin as ferric iron does to ferrous iron.

6. Carboxyhemoglobin behaves just like reduced hemoglobin. The hemoglobin recovered is carboxyhemoglobin.

7. Prolonged action of acid or other agents of denaturation on hemoglobin decreases the percentage of reversion.

From the above findings it is concluded that hemoglobin undergoes two kinds of change when subjected to denaturation. One is reversible and the other is irreversible. The two changes overlap when hemoglobin is heated or is treated with alkali or alcohol. But when hemoglobin is treated with acid the velocities of the two changes are so different that they can be separately studied.

Since the reversible denaturation, which we propose to call pseudo-denaturation, involves only the prosthetic group, it is a reaction peculiar to hemoglobin and is fundamentally different from the denaturation of proteins in general.

This is a preliminary paper.

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[1] Anson, M. L., and Mirsky, A. E., *J. of Physiol.*, 1925, lx, 50.

[2] Anson, M. L., and Mirsky, A. E., *J. of Gen. Physiol.*, 1925, ix, 169.

[3] Roaf, H. E., and Smart, W. A. M., *Biochem. J.*, 1923, xvii, 579.

[4] Quagliariello, G., *Arch. sc. Biol.*, 1923, v, 193.

[5] Conant, J. B., and Scott, N. D., *J. of Biol. Chem.*, 1926, lxix, 575.

3528

The Rôle of the Platelets in Blood Clotting.*

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From the Department of Medicine, Peking Union Medical College.

Blood was obtained from a dog by the method of Lim,* which absolutely excludes admixture of tissue juice. It was kept in contact only with paraffin, was immediately cooled and centrifuged. By differential centrifuging platelets were obtained free of red and white cells. They were washed well in saline and then dissolved in water. This platelet solution was tested for thrombin, prothrombin cephalin and tissue fibrinogen. On treating it with active cephalin and calcium and letting the mixture stand for 15 minutes at room temperature, it was unable to clot a citrated solution of pure blood fibrinogen. A control mixture, using purified prothrombin from fresh blood plasma instead of platelet solution, gave very active thrombin, and clotted fibrinogen in 10 seconds. The platelet solution was capable of replacing cephalin in the activation of the prothrombin solution. This property was unaffected by boiling. It would also directly clot pure blood fibrinogen when free calcium was present, just as well as would an active tissue fibrinogen solution. This action was lost on boiling.

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* A detailed report of this work and of Dr. Lim's method will appear in the July number of the *Chinese Journal of Physiology.*

Platelets, therefore, do not contain thrombin or prothrombin, but do contain free cephalin and tissue fibrinogen, and are thus similar to other body tissues.

This is a preliminary report.

3529

Poisoning by Sikimitoxin.

TSAN-QUO CHOU. (Introduced by B. E. Read.)

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Chinese Bastard Anise, Mang T'sao, commonly known under the colloquial name T'zu Ta Liao, and identified as the fruit of *Illicum religiosum, Sieb.*, has long been known to be poisonous. It is chiefly used as a condiment in preparing Chinese meat dishes. Fatal poisoning by these seeds is not an uncommon occurrence.[1] The chemical nature of its toxic principle was first investigated by Eykman[2] who, working with the Japanese anise, or sikimi-no-ki, also identified as *Illicum religiosum, Sieb.*, isolated from the seeds a crystalline body which he named "Sikimin" and claimed for it a powerful toxic action. 12.5 mgm. of Sikimin killed a young dog in 3 hours. This crystalline body or sikimin, has never since been isolated by other workers; crude seeds, or aqueous extracts, being generally employed for the physiological study of this poisonous plant.[3, 4, 5]

In a recent chemical study of Mang T'sao, the writer isolated from the seeds an amorphous poisonous principle with definite chemical and physical characters. Given to cats, 0.2 mgm. of this amorphous substance per kilogram of body weight was found to be fatal, less than 0.1 mgm. of substance per kilogram of body weight was sufficient to produce the same poisoning symptoms as those produced by the crude seeds, *i. e.*, salivation, vomiting defecation, convulsion and death. The name *"Sikimitoxin"* is suggested for the new toxic principle to distinguish it from Eykman's Sikimin. from which it differs in its physical and chemical properties, and also in its much higher toxicity.

Properties of Sikimitoxin. It is a feebly acid nitrogen-free body and occurs as a white amorphous powder without definite melting point. When slowly heated, it sinters at 63° C., then gradually increases its volume as the temperature rises, and finally becomes a clear liquid at 135° C. It is easily soluble in cold water, chloroform and alcohol, less so in ether and benzene and insoluble in petroleum ether. When brought into contact with a small quantity of cold water, it becomes at once an oil and then gradually dissolves. Its toxicity is rapidly destroyed by the action of caustic alkalies, and greatly decreased by the prolonged action of boiling water with or without the presence of hydrochloric acid.

It is questionable whether Eykman's sikimin was the original substance present in the seeds, or the resulting product formed by the prolonged action of acetic and hydrochloric acid used in his process of isolation, as Sikimitoxin was found to be sensible towards the action of these chemical reagents.

Details concerning the process of isolation, properties and the toxicity of Sikimitoxin will appear later.

This is a preliminary report.

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[1] Read, B. E., *China Med. J.*, 1922, xxxvi, 303.

[2] Eykman, J. F., *Pharm. J.*, 1880-1881, xi, 1046.

[3] Read, B. E., and Kiang, P. C., *Chinese J. Physiol.*, 1927, i, 15-22.

[4] Guerrero, L. E., *Philippine J. Sci.*, 1916, ii, Sec. B., 203-13.

[5] Chen, K. K., *J. A. Ph. A.*, 1926, xv, 861.

3530

Presence of Type Specific Pneumococco-opsonins in Sera of Animals Naturally Resistant to Pneumococcus Infection.

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From the Department of Medicine, Peking Union Medical College.

In a previous communication, Robertson and Sia[1] found that the sera of animals, such as cat, dog, sheep and pig, that are naturally resistant to pneumococcus infection, contained normal opsonins for the pneumococcus, whereas the sera of susceptible animals did not possess such opsonic power for pneumococci virulent for the species. In the course of that study it soon became evident that the serum from a resistant animal, under proper conditions, could be shown to opsonize pneumococci belonging to any of the various serological groups. The question whether the sera of naturally resisant animals contain a common pneumococco-opsonin for all pneumococci, or separate opsonins for each type of the organism immediately arose. The results of Bull and McKee[2] in demonstrating the existence of separate protective substances in normal chicken serum for each type of pneumococcus, together with the author's findings, reported in a previous paper,[3] on the specific effect of pneumococcus soluble substance on the growth of pneumococci in normal serum-leucocyte mixtures, suggest that the sera of resistant animals may contain separate pneumococco-opsonins, specific for each type of pneumococcus. The present report deals with a study which has in view the elucidation of this point.

In this work, sera from two species of naturally resistant animals were used, namely, cat and pig. The method consisted of absorbing the sera by one of the three fixed types of pneumococcus and then testing the opsonic content of the absorbed sera for all the three types. The percentage of polymorphonuclear leucocytes taking part in phagocytosis was recorded and compared with that of control sera which were similarly treated except that no pneumococci were added.

The results thus obtained were very striking. It was found that the absorbed serum lost all of its opsonins for the particular type of pneumococcus employed in the absorption, while those for the other two fixed types were very little affected. Occasionally, evidence of a certain degree of non-specific adsorption was present, but the reduction was never very great. In almost all instances, the absorbed sera showed a hundred per cent reduction in their opsonic content for the type of pneumococcus used in absorption. Spontaneous phagocytosis was practically absent in all the experiments. This was made possible by frequently passing the organism through mice.

Several other experiments were also carried out in which the serum was absorbed by one type of pneumococcus and later reabsorbed by a second type. The serum thus treated still showed a considerable quantity of opsonins for the third type.

The results, therefore, indicate that there are separate type specific pneumococco-opsonins in the sera of animals, namely cat and pig, which are naturally resistant to pneumococcus infection. These findings would also support the idea that natural opsonins are specific.

This is a preliminary report.

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[1] Robertson, O. H., and Sia, R. H. P., Trans. 6th Congress, Far Eastern Association of Trop. Med., 1925.

[2] Bull, C. G., and McKee, C. M., *Am. J. Hyg.*, 1921, i, 284.

[3] Sia, R. H. P., *J. Exp. Med.*, 1926, xliii, 633.

Missouri Branch.

St. Louis University School of Medicine, March 30, 1927.

3531

The Determination of Guanidine Bases in the Blood.*

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Recently, Pfiffner and Myers,[1] in a preliminary report, gave a tentative procedure for the determination of the guanidine bases in blood. In an attempt to apply this method it was found that recoveries of added guanidine in amounts as small as 1 mg. per 100 cc. of blood could not be made. The main difficulty in the determination of these small amounts is the fact that the reagent proposed by Marston[2] does not give a sufficient color production with guanidine in amounts smaller than 0.05 mg. per 5 cc. of solution. It was found that by replacing potassium ferrocyanide with ferricyanide, and eliminating the hydrogen peroxide, a reagent is produced which retains practically all the advantages of Marston's reagent, and in addition gives almost twice as much color with guanidine.

The reagent is made by mixing equal volumes of 10 per cent solutions of sodium nitroprusside, potassium ferricyanide, and sodium hydroxide and diluting the mixture with three volumes of water. The color changes from a dark red to a pale yellow in about 20 minutes and is then ready for use. This prepared reagent will usually keep for 12 hours, but if turbidity develops a fresh reagent should be made up. It is used in the proportion of 1 cc. of the reagent to every 5 cc. of the solution to be tested.

Equal amounts of guanidine or methyl guanidine give the same color value with the reagent, whereas dimethyl guanidine gives only two-thirds as much color. The color is an orange red, which develops to its maximum in 2 minutes. Creatine gives 1/10 as much color as does guanidine, and the color develops to its maximum in about 5 minutes.

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* This work was begun in the Department of Biological Chemistry of St. Louis University, but concluded at the University of Kansas.

With creatinine and urea the color develops very gradually and after standing with the reagent for 10 minutes, urea gives 1/250, and creatinine 1/100 as much color as does guanidine. Guanidine gives its full color development in the presence of any of the above substances, and in their presence the amount of guanidine may be determined by subtracting the color given by these substances. One mg. of uric acid, 1 mg. NH_3-N, and 40 mg. of sodium chloride per 5 cc. of solution cause a 25 per cent reduction in the color given by guanidine. If the above are present together in 5 cc. of solution, a loss of approximately 75 per cent in color is obtained.

A large number of blood determinations have been made by extracting the residue obtained by evaporating the Folin-Wu filtrate with absolute alcohol, as carried out by Pfiffner and Myers, except no provision was made for the removal of urea. The disadvantages of this method were the inability to obtain good recoveries of added guanidine with amounts less than 1 mg. per 100 cc. of blood, and a turbidity in the final solution which was very difficult to remove. This method was abandoned when it was found that norit or blood charcoal would quantitatively adsorb the guanidine bases from a slightly alkaline solution, and that they could again be practically completely removed by treating the charcoal with acidified alcohol. Urea is not absorbed.

The method for blood is as follows: 50 cc. of the Folin-Wu blood filtrate is made slightly alkaline by the addition of 0.3 cc. (from 3 to 4 drops) of 10 per cent sodium hydroxide. 0.5 gm. of blood charcoal (Merck's purified by acid) is added and brought into suspension by shaking. After two minutes the mixture is filtered, care being taken to prevent loss of charcoal, and the flask drained as completely as possible. The filter is allowed to drain for five minutes and is then placed in the same flask from which the filtration was made. Twenty-five cc. of 95 per cent alcohol containing 0.5 cc. of normal hydrochloric acid is added to the flask, the flask corked and shaken to bring the charcoal in suspension. The charcoal is permitted to remain in contact with the alcohol over night and is then filtered off. Twenty cc. of the filtrate is evaporated over a water bath at about 90 degrees, evaporation being hastened by means of a fan. If the evaporation is carried out over a boiling water bath, the residue is very dark and interferes with the color comparisons. The residue is dissolved in 5 cc. of water and 1 cc. of the nitroprusside reagent is added. A slight flocculent turbidity forms at this point, which can be very easily removed by centrifuging. The reading is made within ten minutes against an appropriate standard of guanidine carbonate. The standard is made up in 10 cc. volume to which 2 cc. of the nitroprusside reagent is added.

By means of this method our recoveries average 80 per cent and have never varied more than 5 per cent either way. Amounts as small as 0.2 mg. of guanidine or methyl guanidine, when added to 100 cc. of blood, may be determined.

In the application of this reagent to normal and pathological blood, the creatine content should be taken into account and a correction applied. In a series of creatine additions to blood to determine this correction we found that for every milligram of creatine, 0.07 mg. of guanidine should be subtracted from the guanidine reading. Thus, if a total reading is 1 mg. of guanidine and the creatine is 4 mg., the figure 4 times .07 equals .28, must be subtracted from 1 mg. The correct reading then will be .72 mg. Normal bloods with this correction applied give a reading equivalent to a guanidine content of from 0.07 to 0.15 mg. per 100 cc., while in a few cases of hypertension a content of between 0.3 and 0.6 mg. was noted. Pfiffner and Myers report in one case of hypertension without nitrogen retention a guanidine content of 10 mg., and a like value in a case of chronic nephritis. We have not encountered any such high values. It must be emphasized that this colorimetric method is not absolutely specific for guanidine, and that the increases in color development may be due to other undetermined substances.

An application of this method for the determination of guanidine in the urine is being studied, the details of which will be given in a later paper.

This is a preliminary report.

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[1] Pfiffner, J. J., and Myers, V. C., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 830.

[2] Marston, H. R., *Austral. J. Exp. Biol. and Med. Sc.*, 1924, ii, 57.

3532

The Isoelectric Point and the Median Minimum of Growth.

CLIFFORD H. FARR. (Introduced by Leo Loeb.)

From the Department of Botany, Washington University, St. Louis.

The typical curve which has been secured for growth of organisms in varying hydrogen ion concentrations is platycurtic and bimodal. Jacques Loeb[1] secured similar curves for the viscosity, osmotic pressure, potential difference, and swellability of gelatin and other proteins. He explained the bimodality of these graphs upon the basis of the Donnan's equilibrium, and arrived at the conclusion that the median minimum lying between the acid and the alkaline optima respectively is located at the isoelectric point of the protein in each case. In physico-chemical investigations, then, one seems quite warranted in regarding the median minimum of curves plotted along the pH scale as a sound criterion of the isoelectric point of the protein involved. A number of biologists, including Robbins[2] and Pearsall,[3] have undertaken to utilize this relationship in the interpretation of similar graphs obtained in the study of plants and animals, that is, concluding, for instance, that the median minimum for growth curves is an index of the isoelectric point of the constituent proteins.

Michaelis[4] has pointed out that "the isoelectric point is not deflected by true salt formation" (p. 145). If the median minimum of biological graphs plotted along the pH scale is at the isoelectric point of the proteins composing the tissue concerned, then it would seem that this medium minimum should be located at approximately the same hydrogen ion concentration irrespective of the molar concentration of the salt solution bathing the tissue. If, on the other hand, the median minimum is found to shift toward the acid or the alkaline side with alteration of the salt concentration, there would be definite evidence that the median minimum of these biological curves is determined by some other factor than the isoelectric point of the organism.

Some experiments, which are to be reported in full elsewhere,[5] throw some light upon this question. The rate of elongation of the root hairs of Georgia collards, a variety of *Brassica oleracea,* growing in solutions of calcium chloride of various hydrogen ion and molar concentrations, has been measured. The increase in size of these root hairs can be determined very accurately, inasmuch as they are single cells, which enlarge in only one dimension, and have very definite termini. Furthermore, they will grow in calcium hydroxide alone, and in all concentrations of calcium chloride below a certain maximum, approximately 0.185 M. Data has been obtained upon the rate of growth of about 700 of these root hairs in a total of 49 solutions of different concentration, over a period of three hours in each case. Curves for variations in rate in different hydrogen ion concentrations were plotted for calcium hydroxide, and for calcium chloride at 0.008, 0.020, 0.028, 0.060, and 0.120 mols. respectively. In all cases except the last-named concentration the curves were bimodal.

The data thus obtained may be plotted in a graph of three dimensions: the ordinates, being rate of growth; the two abscissae, being hydrogen ion concentration and molar concentration of the salt respectively. When this is done, it becomes apparent that the median minimum, which begins at pH 9 in calcium hydroxide, shifts with increasing salt concentration to pH 8 in 0.020, pH 7.4 in 0.028, pH 6.4 in 0.060. No median minimum occurs in 0.120 M. inasmuch as it, by projection, is found to lie outside of the concentrations in which the root hairs will grow, that is, in too acid a solution for the amount of calcium present. Not only does the median minimum shift toward the acid side with increased salt concentration, but the acid optimum, the alkaline optimum, the alkaline limit, and, at low concentrations, acid limit do likewise.

From this study it may, therefore, be concluded that the median minimum of some growth curves, at least, are determined by some other factor than the isoelectric point of the constituent proteins alone. The latter may be one of the factors involved, but it can hardly be the sole consideration. It is apparent that the median minimum in shifting toward the acid side, will in all probability intersect the hydrogen ion concentration of the isoelectric point; so that, at one salt concentration the two will be identical. The finding that they are identical in a given solution is not, however, to be taken as evidence that they are necessarily related to each other.

This is a preliminary report.

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3533

Method for Study of Formation of Amino Acids in Animal Organism.

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The rate of growth of young white rats on a diet containing sodium benzoate has been used as an index of the rate of formation of the amino acid, glycine, which is required for the detoxication of benzoic acid, as well as for the synthesis of new tissue proteins. Normal growth occurred on a diet containing 2 per cent sodium benzoate and 12 per cent casein, but the rats failed to grow on diets containing 3 per cent sodium benzoate and 12, 35, or 50 per cent casein. The addition of one equivalent of glycine (1.56 gm.) to the diet containing 12 per cent casein and 3 per cent benzoate resulted in normal growth. In these experiments, 38 gm. of casein did not successfully replace 1.56 gm. of glycine, and it was, therefore, concluded that casein was unable to supply precursors of glycine. This investigation of the formation of amino acids in the animal organism is being continued and extended.

This is a preliminary report.

3534

Sympathetic and Parasympathetic Responses after Intradermal and Intracorneal Injections

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In a recent series of papers Luithlen and Molitor[1] have reported that following an intradermal or intracorneal injection of 0.1 cc. of 0.9 per cent NaCl solution in a cat, the response of the vagus either to an electrical stimulus or to a parasympathetic-stimulating drug (cholazyl) is greatly increased. Both vagus trunks were cut, the electrical stimulus being applied peripherally; the drug was injected intravenously. They report that they were able to get a constant series of control responses to electrical or drug stimulation before the intradermal or intracorneal injection. They find that the enhancing effect is not obtained when the intradermal injection is made into a denervated area and conclude that it is due to a reflex heightening of vagus sensitivity, the afferent path being afferent fibers stimulated by the injected solution. It is difficult to see how peripheral manifestations of reflexly increased vagus tone can be elicited when both vagus trunks are cut. These workers also report an increased response to adrenalin after intradermal and more especially after intracorneal injection, attributing this to a reflexly increased sympathetic sensitivity. The increase in sympathetic response was less nearly constant than the increase in the parasympathetic response.

Since these results were so surprising it seemed worth while to repeat these experiments. The precautions prescribed by Luithlen and Molitor as essential to success were carefully observed, namely, proper preparation of the site of intradermal injection, maintenance of body temperature, anesthesia, etc. Shielded electrodes were used for nerve stimulation, the source of current being a chloride accumulator. The drug injections were made into a femoral vein, the injection time being 10 seconds. Arecoline was the parasympathetic drug employed, adrenalin the sympathetic. Adrenalin dilutions were made from a 1-1000 Parke-Davis preparation, no dilution more than 12 minutes old being used. A threshold electrical or drug stimulus was established, the criterion for parasympathetic stimulation being a just perceptible fall in blood pressure, that for sympathetic stimulation (by adrenalin) a just perceptible rise. One-fifth to one mgm. of atropin sulphate was given intravenously before the adrenalin injections, to abolish any possible increase of vagus response from the intradermal or intracorneal injection which might mask an increase of sympathetic response. The pressor effect of such small doses of adrenalin is of course followed by a marked depressor effect. No change in blood pressure resulted from the intradermal or intracorneal injections themselves. In a given experiment the interval between successive electrical or drug stimulations was constant, being 3 minutes in some cases, 5 in others.

Satisfactory records were obtained on 13 cats. After the threshold stimulus was established a series of 5 to 10 such stimuli at constant intervals was given, the intradermal or intracorneal injection then made and the series of threshold stimuli continued. Our results may be expressed briefly by the statement that no greater changes in the responses to the stimuli following an intradermal or intracorneal injection were obtained than could occur spontaneously in the control series. Usually a quite constant series of responses to a series of equal drug and as nearly as possible equal electrical stimuli was obtained. On one occasion, however, an electrical stimulation of the vagus of apparently the same strength as its predecessors evoked a markedly greater response, without any intradermal or intracorneal injections having been made. And on only one occasion did the vagus response to electrical stimulation increase markedly after intradermal injection. In all the other cases the vagus response to electrical stimulation was unaltered by an intradermal or intracorneal injection. In no case was the vagus response to arecoline or the sympathetic response to adrenalin affected by the intradermal or intracorneal injection. We are thus unable to confirm Luithlen and Molitor's findings of an increased sympathetic and parasympathetic response after intradermal or intracorneal injections of 0.1 cc. of 0.9 per cent NaCl solution.

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3535

The Action of Mineral Oil per Os on the Organism.

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Leitch[1] has shown that mineral oils applied repeatedly to the skin of animals induce cancer. It is known that these same oils are responsible for cancers in man. This is well illustrated by the studies of the so-called "mule spinners cancer" and the prevalence of cancers of the skin among the workers of the paraffin mines of Scotland. Several paraffin cancers have also been described in America and in other parts of Europe.[1]

Our studies have shown that many animal and vegetable oils[2, 3] are not absorbed when introduced under the skin of animals, but produce lesions quite identical to those produced by various mineral oils and tar.[4, 5]

Our analyses of cancer have shown that it is directly the result of the removal of the excess of the ergusia (fat soluble vitamins) from local areas of the tissues.[6] Such a removal of this lipoid substance or substances leads to a disappearance of the blood vessels, a crowd-ing of the cells and stagnation sufficient for their independent growth. Mineral oils, tars, and especially the animal and vegetable oils and fats, which are largely free from fat soluble vitamins, when introduced into the tissues, induce cancer and crowd the cells in that they are able to dissolve and remove the fat soluble vitamins from adjacent areas of the tissues.[7]

Since pastries and manufactured vitamin-free fats are common articles of diet of Europeans and Americans, and since cancer of the stomach is very prevalent in these parts of the world, and not so common in many other parts[8, 9] it became of interest to investigate these fats and oils as a possible cause of cancers of the gastrointestinal tract. Mineral oils, when ingested, pass directly through the entire length of the gastrointestinal tract. Vegetable and animal oils pass undisturbed through the esophagus and stomach. They are then absorbed from the intestinal canal and either burned or stored in the body.

In the light of these facts, it became evident that fats and oils of all kinds, which are free from fat soluble vitamins, may affect the intestinal canal when given in the food, as they are known to affect the skin when applied to it. In the intestinal canal they should not only tend to remove the fat soluble vitamins from the intestinal wall and cause precancerous and cancerous lesions, but they must also remove these same vitamins from other articles of food with which they come in contact during chewing, swallowing and digestion.

Different oils should act differently in this regard. The animal and vegetable oils should cause such changes only in the mouth, esophagus and stomach. When absorbed from the intestines and burned they should give these vitamins back to the body. The use of such oils should have no effect, therefore, upon the general nutrition of the animal ingesting them, but should act only to cause changes in the upper digestive tract before they are absorbed. These effects should be most marked in the places where they are brought into closest contact with the wall of the digestive tube.

Mineral oils, on the other hand, must affect the entire alimentary tract and since they are not absorbed to any extent, but are eliminated with the feces, they should tend also to strip the food taken by the individual of its fat soluble vitamins, and cause nutritional disturbances in this individual.

It had become of interest, therefore, to test for the effect of various of these oils upon the gastrointestinal tract of rats and mice, and also upon the growth and general health of these animals. Since the results of our first experiments have been so striking, it seemed important to report them at once, especially on account of the wide use of vitamin free oils and greases at the present time, and the extensive use of mineral oils as salves, in nose and throat conditions, in X-ray diagnosis, and as a purgative. Young rats were used for these experiments. The effect upon the growth of these rats of various oils in different diets, are recorded in the curves of Fig. 1. In order to make our data more quantitative, the rats were fed on a balanced dietary of known chemical and vitamin content. To compare the effect of the oils with effects resulting from an absence of fat soluble vitamins, other rats were fed on a dietary deficient in these vitamins. In these first experiments, crisco, nujol and heavy paraffin oil have been tested.

In the first experiment, M-1, four rats were fed on a diet deficient in vitamin A:

Potato starch,	80 grams.
Egg albumen crystals,	50 grams.
Salts (McCollum),	10 grams.
Crisco,	30 grams.
Vegex,	10 grams.

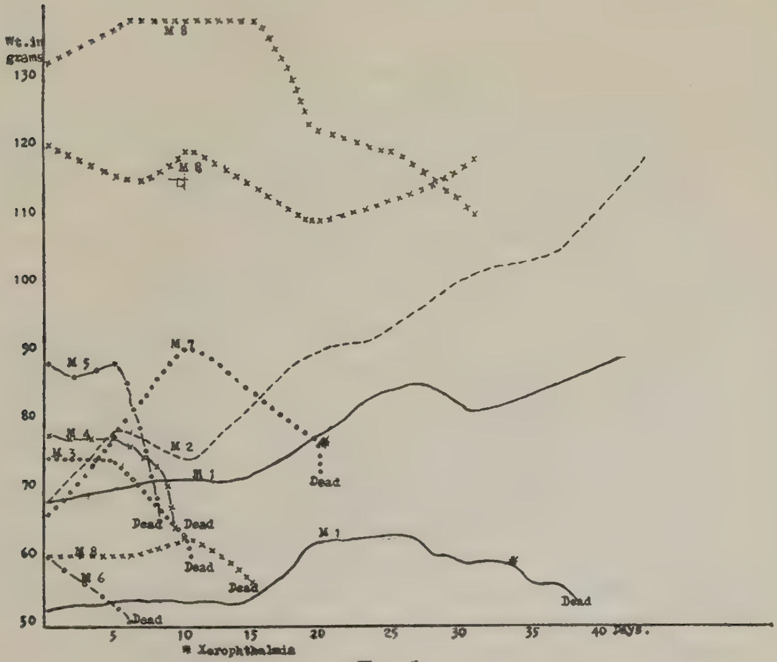

FIG. 1.

Growth curves of rats fed various dietaries with and without vegetable fats and mineral oils.

The growth curves of these rats are typical of other curves of rats fed on diets deficient in vitamin A. Two of them are included in Fig. 1, curve M-1.

The rats in Cages M-2, M-3, M-5 and M-6, were given the balanced dietary:

Potato starch,	80 grams.
Egg albumen crystals,	50 grams.
Salts (McCollum),	10 grams.
Butter,	30 grams.
Vegex,	10 grams.

Four rats eat twice this quantity each week. In Cage M-3, thirty grams of crisco (or about 14 per cent of the total diet) was added to the quantity of diet given above. Shortly after this diet was given, these rats showed fat in the stools and lost weight. Later this fat disappeared and they have gained normally since that time. The growth curve of one of these rats is Curve M-2, Fig. 1.

Thirty grams of nujol (or about 14 per cent of the total diet) was added to the balanced diet as given above, and fed to rats in Cage M-3. These rats suffered early from diarrhea and died after 10 days (Curve M-3, Fig. 1). A similar result was obtained from the study of rats in Cage M-5, where 15 grams of nujol (about 7 per cent of the total diet) was added to the balanced dietary. These rats died in 7 days (Curve M-5, Fig. 1). In Cage M-4, thirty grams of nujol was added to the minus vitamin A dietary, rather than to the balanced ration. This change had no effect. These rats lived as long as the others. They died in about 9 days. (Curve M-4, Fig. 1). In Cage M-6, forty-five grams of nujol (20 per cent of the whole dietary) was added. The destruction of these rats was immediate. The rats died in 6 days (Curve M-6, Fig. 1).

During these months of late winter and early spring, the butter is not so rich in fat soluble vitamins as at other times. In Cages M-7 and M-8, the rats were fed on the balanced diet, plus 2 per cent cod liver oil. In Cage M-8, five grams of heavy petrolatum (about 2.6 per cent of the total dietary) was added to the new balanced dietary in the proportions given above. The growth curves of two older and one younger rat of this series are included in Fig. 1, M-8. It is interesting to note that while the older rats withstood the action of the oil better than the younger ones, they also either do not gain or lose weight after a short time.

In Cage M-7, an attempt was made to administer what has been found to be a therapeutic dose per kilogram body weight for children. Two and a half grams of heavy petrolatum (about 1.3 per cent of the total dietary) was added to the new balanced dietary given above. These rats have died in less than one-half the time of the rats on a minus vitamin A dietary (Curve M-7, Fig. 1).

The rats in all the cages, excepting where the very small doses of mineral oil were given, died without showing any xerophthalmia. In Cage 7, two rats showed for a short time before death the narrowing of the pupils and clouding of cornea, which is typical of this affliction.

As is evident, therefore, the mineral oils, quite different from the vegetable oils, not only remove the fat soluble vitamins from the food, but produce other toxic effects as well. From our studies of the effects of these oils on the tissues and in the production of cancer, it may be assumed that their toxic effect is due to the fact that they act on the mucous membrane of the intestines to further strip these tissues of the body of their fat soluble vitamin reserve.

This is a preliminary report.

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[4] Jorstad, L. H., and Johnston, C. G., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 86.

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[6] Burrows, M. T., *J. of Can. Res.*, 1925, x, 239.

[7] Jorstad, L. H., *J. Exp. Med.*, 1925, xlii, 221.

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3536

Studies on Fatty Infiltration of the Liver Induced by Lipoid Solvents.

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In a previous paper, Johnston and the author[1] reported that fatty infiltration of the liver occurs a certain period of time after the injection of a mixture of mineral oil and coal tar into the subcutaneous tissue of rats fed a diet rich in vitamin A. We thought at that time that this mixture of lipoid solvents alone produced the fatty infiltration in these animals. More recent studies have shown that this is not true. It was a time factor only which brought about the action of the mixture of mineral oil and coal tar. Later experiments have shown that other lipoid solvents will produce the same effect in the liver after a longer interval of time. A mixture of coal tar and paraffin produces the change 30 days after the injection, coal tar in 90 days, mazola oil in 60 to 90 days, mineral oil in 45 to 60 days, and a mixture of coal tar and mazola oil in 40 to 60 days. Thus, the time of occurrence of the fatty infiltration is governed by the particular kind of lipoid solvent used. At the times given above there is an extensive infiltration involving the entire parenchyma of the liver. Previous to this time in certain of the above series a few scattered areas of vacuolization were found in a few of the livers.

The infiltration does not occur in the animals fed a ration deficient in vitamin A, and only to a slight degree in the animals fed a well balanced stock ration. It does not occur in rats fed a rich vitamin A dietary that are not injected with the substances listed above. These substances are lipoid solvents. It is in a system saturated with ergusia (vitamin A) that these lipoid solvents induced the transportation of fat to the liver. As Burrows[2] has shown with the tissue culture, the migration of fat is a simple process. The fat droplet migrates from an area poor to one rich in ergusia. In these terms fatty infiltration can be understood as being due to a disturbance in the ergusia balance of the system induced by the introduction of a lipoid solvent into it. The same amount of fat is included in the rations deficient in vitamin A, but the only ergusia present in these animals is that stored by the body previous to the beginning of the deficient vitamin A diet.

It is well known that chloroform causes a necrosis of the liver. Certain authors have reported cases of fatty infiltration following chloroform inhalation. The protective action of a high carbohydrate, low fat dietary against chloroform poisoning has been emphasized by many authors.[3] In our studies we find that chloroform (2 cc. subcutaneously or a small amount by inhalation) causes an extensive necrosis of the liver within 7 days, and on a fat diet deficient in carbohydrate, the lesion occurs within 24 to 36 hours. With the dietary high in carbohydrates the lesion is less extensive and a certain number of the livers show a vacuolization associated with the necrosis. However, this vacuolization occurs in areas that have undergone a certain degree of necrosis. The cells in these areas are granular and the nuclei are centrally placed. In the animals injected with the lipoid solvents mentioned above, the nucleus is peripherally placed as if pushed to one side by the fat taken up by the cell. The cells are not granular. This does not mean necessarily that chloroform acts differently, but probably only more drastically than coal tar, paraffin and mazola oil, or mixtures of them.

This is a preliminary report.

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[1] Jorstad, L. H., and Johnston, C. G., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 86-88.

[2] Burrows, M. T., *Am. J. Anat.*, 1926, xxxvii, 2, 289-349.

[3] Opie, E. L., and Alford, L. B., *J. Am. Med. Assn.*, 1914, lxii, 895.

3537

Contractures Caused by Tenotomy and by Tetanus Toxin.

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The term, contracture, is here used to designate a state of permanent shortening of a muscle which persists after section of the motor nerve or after death of the animal. Since under these conditions no nerve impulses can reach the muscle it is obvious that when once developed this sort of shortening is independent of the nervous system and is due to a more or less permanent alteration of the muscle. These contractures develop too quickly to be due to fibrosis and histological sections show no increase in connective tissue. The muscle fibers themselves have acquired a new and shorter length.

When a resting muscle is made to support a moderate load it undergoes in addition to the elastic stretch, which disappears promptly when the load is removed, also a more or less permanent elongation (Nachdehnung of Blix,[1] permanent deformation of Langelaan[2]). In the contractures caused by tendon section and by tetanus toxin the elasticity remains approximately normal but the resistance to permanent elongation is greatly increased.

In addition to its action on the spinal cord, tetanus toxin also exerts a direct action on muscle, causing a shortening and increased resistance to permanent elongation when injected after section of the motor nerve.

This is a preliminary report.

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[1] Blix, M., *Sk. and Arch. f. Physiol.*, 1892-95, iii, 295; iv, 399; v, 150.

[2] Langelaan, J. W., *Brain*, 1915, xxxviii, 235.

3538

Injections of Caffeine and Adrenalin and Subsequently Staphylococcus Aureus Suspensions on Myocardium and Endocardium.

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Fleisher and Loeb[1] have shown that a single intravenous injection of a mixture of .025 gm. caffeine sodium benzoate and .2 cc. adrenalin chloride causes a gross myocardial lesion in the left ventricle and papillary muscle of the left ventricle in rabbits, in about 60 per cent of cases. We have confirmed their observations, and have in addition observed a lesion in the left auricle which follows these injections in some cases. In order to rule out the possibility that these may be spontaneous lesions, and in order to analyse the functional effects of these myocardial changes, a series of rabbits was examined electrocardiographically before and after these injections.[2] We may only state here that the cardiac condition is not the result of previous spontaneous myocardial disease.

Experiments were carried out to determine whether this lesion produced by caffeine and adrenalin injections would render the rabbit's heart more susceptible to abscess formation when, subsequently, a pyogenic organism in culture is injected intravenously. For this purpose 24 rabbits were injected with a single dose each of the caffeine and adrenalin mixture. Then at varying periods afterwards, each was injected with 1 cc. of a 48 hour bouillon culture of a laboratory strain of living *Staphylococcus aureus* of a sufficient virulence to cause the production of abscesses in the kidneys in a large number of cases without causing the death of the animals within the following three weeks. The rabbits were killed at varying periods following these injections and the organs examined grossly and microscopically. Twenty-three of the 24 animals showed the gross myocardial lesion which usually follows caffeine and adrenalin injections. In addition, 8 had abscesses in the myocardium. Of these 8, 6 showed a single abscess in or in very close proximity to the caffeine and adrenalin lesion in the myocardium of the left ventricle. The other 2 rabbits had both an abscess in or around the caffeine and adrenalin lesion, and in addition one showed an abscess in the normal myocardium near the apex of the left ventricle, and the other vegetation on the mitral valve. Of these 23 rabbits 3 showed gross endocardial lesions, which consisted in 2 cases of a mural thrombus adherent to the endocardium of the left ventricle adjacent to the lesion produced by caffeine and adrenalin in the myocardium of the left ventricle. The third case, previously described, showed an ulcerated vegetation at the line of closure of the mitral valve.

Eighteen control rabbits received the same dose of *Staphylococcus aureus* culture intravenously without previous injections of caffeine and adrenalin. Only one of these animals showed an abscess in the myocardium, and this rabbit probably had a preexisting spontaneous myocardial lesion, the site of which was also the site of the abscess formation. Furthermore, none of these showed an endocardial lesion.

However, it was found that when a more virulent strain of *Staphylococcus aureus* was used, the rabbits which received merely *staphylococcus aureus* injections also showed myocardial abscesses. Thus in a second experiment in 10 out of these 13 control rabbits such lesions developed. Those animals which had been injected with caffeine and adrenalin and, subsequently, with the more virulent staphylococcus culture, had myocardial abscesses in 10 out of 11 cases. While in this experiment the difference in the number of abscesses in the two classes of rabbits is very slight as compared with the results obtained in the first experiment in which the less virulent strain of staphylococcus was used, there was a definite difference in the localization of the abscesses between the control animals and the caffeine and adrenalin animals. The myocardial abscesses in those animals which had received caffeine and adrenalin in addition to the staphylococcus culture were in most cases situated in or around the caffeine and adrenalin lesion in the left ventricle; while in those which had received only injections of staphylococcus culture the abscesses were more frequent in the right ventricle, and practically never found at the site of the lesion produced by caffeine and adrenalin.

We concluded, therefore, that the myocardial lesion in the rabbit produced by the injection of adrenalin and caffeine influences the localization of abscesses caused by the intravenous injection of staphylococcus cultures, and that under certain conditions these myocardial lesions may also increase the number of abscesses.

This is a preliminary report.

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[2] Becke, W. G., and Johnson, Scott, *Am. Heart J.*, 1927.

3539

The Effect of Follicular Extract on the Generative Organs of Hysterectomized Guinea Pigs.

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The corpus luteum has the following functions: (1) It sensitizes the uterine mucosa in such a way that mechanical stimuli applied to the sensitized tissue cause the production of placentomata, or the embedding of the ovum calls forth the production of maternal placenta. The corpus luteum also produces a slight predecidual proliferation in the mucosa.[1] (2) The corpus luteum induces a proliferation of the mammary gland,[2] and (3) The corpus luteum prevents ovulation.[3]

There are certain changes which occur in the sexual cycle previous to the development and functioning of the corpus luteum, namely, hyperemia and occasional mitoses in the mucosa of the uterus, a slight proliferation of the mammary gland and a marked proliferation of the vaginal epithelium. These latter phenomena are, therefore, not produced through the action of the corpus luteum, but they must be due to the influence of maturing or mature follicles, the development of which coincides with the occurrence of these latter changes. We divided, therefore, the sexual cycle into two phases, (1) the follicular phase and (2) the lutein phase, both of which are dominated by distinct hormones, although in certain respects their effects are similar.[4] Subsequently the experiments of Frank[6] and especially of Allen and Doisy,[5] and later, of many others proved that the contents of ovarian follicles produce certain changes in the sexual organs very similar to those observed during the follicular phase of the cycle. However, Allen, as well as Frank and others, on the basis of their observations, went so far as to identify the follicular hormone with the ovarian hormone in general. In their opinion the corpus luteum was merely a place where the follicular hormone was deposited.

These conclusions, according to our opinion, are not in accordance with the facts established during the last 25 years, and we believe that the interpretation of these authors is, in part at least, due to the circumstance that they depended largely on the mouse for the study of the sexual cycle, and used as the method of investigation principally the microscopic examination of vaginal smears devised by Stockard and Papanicolaou.[7] While the latter has the great advantage of simplicity, still it gives a very limited insight into what is actually taking place during the sexual cycle. As for the mouse and rat, their sexual cycle is peculiar in that the lutein phase is here developed only in a very rudimentary fashion. In order to clear up still more definitely the relation between follicular and corpus luteum hormones we turned to the guinea pig, in which both phases of the cycle are well developed and have been analyzed by us thoroughly in previous investigations. In addition, instead of applying the smear method we made a complete microscopic examination of the various generative organs of every guinea pig injected with follicular hormone. These investigations were begun about one and one-half years ago, and they comprise a series of different experiments. We shall limit ourselves in this communication to a consideration of those experiments in which, about 3 to 5 days following the period of heat, we extirpated completely or almost completely the uterus of guinea pigs, and after variable periods of time administered to such hysterectomized animals a series of injections of follicular extract extending over a period of 10 to 14 days. Usually two, but in one case three daily injections of 1 cc. of follicular extract were given to each animal.* At the end of the experiment the vagina, cervix, uterus, ovaries and mammary gland were examined microscopically. The ovaries were always studied in serial sections.

While in the guinea pigs injected during the sexual cycle, and also in immature or castrated guinea pigs the follicular extract caused opening of the vagina, proliferation of the vaginal epithelium, proliferation of the mammary gland, and in some cases hyperemia and an edematous condition of the uterus, in hysterectomized guinea pigs on the other hand, the vagina remained closed except that an occasional slight tear was seen, such as may occur in various conditions, perhaps as the result of injury; the vagina not only did not proliferate but was found to be more inactive than in immature guinea pigs, or even in guinea pigs during the second part of the cycle. The vaginal cells were very vacuolar, in some cases filled with large masses of mucus, and polymorphonuclear leucocytes migrated through the wall of the vagina in such large numbers that small abscesses were produced in the mucosa. The small remnants of the uterine mucosa did not show any predecidual proliferation. These peculiar results can be readily explained if we study the ovaries in such cases. In accordance with our previous experiments, we find that, in the guinea pig, extirpation of the uterus a few days following the oestrus prevents the degeneration of the corpus luteum which would normally occur within the next two weeks; instead, this organ remains alive and functioning for a period extending over at least 2 to 3 months.[8] In consequence of the persistence of the corpus luteum, ovulation is prevented, the vagina no longer opens, the vaginal epithelium becomes vacuolar and large masses of polynuclear leucocytes migrate through it; the sexual cycle is suspended and in certain respects the functioning of the generating system of the guinea pig becomes similar to that of the rabbit. However, the mammary gland under the stimulus of persisting corpus luteum secretion grows to a considerable size. In regard to the character of the vagina and mammary gland the condition in such animals resembles that obtaining in pregnancy. In both pregnant and hysterectomized guinea pigs, furthermore, the corpus luteum does not interfere with the development of the ovarian follicles, some of which mature, while all undergo the typical retrogressive changes in the course of time.

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* In this series we used the preparation made and placed at our disposal by Parke, Davis & Company in Detroit. In other series of experiments we also used a follicular extract prepared by one of us (Kountz). The former preparation was tested in castrated guinea pigs and it was found that 12 injections of 1 cc. each administered on 6 successive days usually produced the characteristic changes.

If we now inject follicular extract of moderate potency twice or three times daily into such pregnant or hysterectomized animals it is not able to counteract the effect of the corpus luteum, while the same injections in other guinea pigs would produce the typical changes. Furthermore it does not prevent the maturation of follicles which normally occurs in hysterectomized as well as in other guinea pigs. This latter fact is in accordance with the conclusion repeatedly emphasized by us that the corpus luteum does not inhibit maturation of follicles but only ovulation; in addition, we now find that it also inhibits the effect of follicular hormone on the vagina.

In this connection it may be stated that the follicular extract in general seems to promote the growth and full development of the follicles, while at the same time it not only does not accelerate, but on the contrary seems to inhibit, ovulation in the guinea pig. In a similar way to the persistent corpus luteum of the hysterectomized guinea pig the persistent corpus luteum of pregnancy likewise prevents the typical proliferative changes in the vagina; instead, this organ shows the characteristic degenerative condition found in hysterectomized guinea pigs. However, in this connection, we must recall the fact that, as we have found previously, there exist complicated conditions in pregnancy inasmuch as in this state certain factors prevent the typical predecidual reaction of the uterus from taking place, in case an ovulation is called forth experimentally and a new corpus luteum is thus made to develop during pregnancy.

In conclusion, we may state that these investigations substantiate the interpretation of the mechanism of the sexual cycle given by us previously as to the existence of two different phases in the cycle; they also confirm our conclusions that in the regulation of the sexual cycle we do not have to deal with one single generative hormone residing in the follicles, but with the complex interaction of at least two and probably an even larger number of substances derived from corpus luteum as well as from follicles.

This is a preliminary report.

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[1] Loeb, Leo, *J. Am. Med. Assn.*, 1907, l, 1809; *Zentralbl. f. allg. Path.*, 1908, xviii, 563; *Arch. Entwicklgsmech.*, 1909, xxvii, 89 and 463; 1911, xxxi, 456; 1911, xxxii, 67.

[2] Ancel, P., et Bouin, P., *J. de Physiol. et de Pathol. Gen.*, 1911, xiii, 31. Loeb, Leo, and Hesselberg, Cora, *J. Exp. Med.*, 1917, xxv, 285 and 305.

[3] Loeb, Leo, *D. Med. Wochens.*, 1911, xxxvii, 17; *J. Morph.*, 1911, xxii, 37. Marshall, F. H. A., and Halnan, E. T., *Proc. Royal Soc.*, Ser. B., 1917, lxxxix, 546.

[4] Loeb, Leo, *Biol. Bull.*, 1914, xxvii, 1; *Trans. Am. Gyn. Soc.*. 1917; *Surg. Gynecol. and Obstet.*, 1917, xxv, 300; *Am. J. Anat.*, 1923, xxxii, 305.

[5] Allen, E., *Am. J. Anat.*, 1922, xxx, 297. Allen, E., Pratt, J. P., and Doisy, E. A., *J. Am. Mèd. Assn.*, 1925, lxxxv, 399. Allen, E., Francis, B. F., Robertson, C. C., Johnson, C., Doisy, E. A., Kountz, W. B., Gibson, H., *Am. J. Anat.*, 1924, xxxiv, 133.

[6] Frank, R. T., *J. Am. Med. Assn.*, 1922, lxxviii, 181; *J. Obstet. and Gyn.*, 1924, viii, 573. Frank, R. T., and Gustavson, R. G., *J. Am. Med. Assn.*, 1925, lxxxiv, 1715.

[7] Stockard, C. R., and Papanicolau, G. N., *Am. J. Anat.*, 1917, xxii, 225.

[8] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 441.

[9] Loeb, Leo, *Biol. Bull.*, 1914, xxvii, 30.

3540

Animal Passage Hypoglycaemia.*

J. B. COLLIP.

From the Department of Biochemistry, University of Alberta.

As the writer has never been able to duplicate the results obtained by him in connection with a study of plant extracts containing a hypoglycemia producing substance, and described as animal passage hypoglycemia,[1] he is now of the opinion that such results must have been due to an organism. The following observations are the basis for this view:

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* This paper received too late to be inserted in its proper order.

1. Attempt to purify the unknown agent in passage blood by the use of alcohol always resulted in failure.
2. The addition of tri-cresol to active passage serum as a preservative resulted in complete loss of activity.
3. The phenomenon has been reproduced on 3 different occasions during the past 3 years by using dried passage blood obtained during the original investigation, although the results have never been as clear cut as formerly obtained.
4. From blood cultures of reacting rabbits Dr. Shaw of the Department of Bacteriology of the University of Alberta has obtained an organism of the nature of a coccus, cultures of which injected into rabbits produce death with hypoglycemia. Necrotic changes of the parenchyma of the liver were also observed. Intravenous injections of such a culture produced at first a marked hyperglycemia, later the blood sugar fell and hypoglycemia was present at death. The results are somewhat similar to those described by Menten and Manning.[2] One fully realizes that such observations do not prove that the original results were due to an organism. The writer feels it his duty, however, to make this statement and to add that he sees no reason to suppose that plant insulin is different from animal insulin. The phenomenon of passage hypoglycemia is something quite apart from insulin action.

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[1] Collip, J. B., *J. Biol. Chem.*, 1923, lviii, 163.

[2] Menten, M. L., and Manning, H. M., *J. Infec. Dis.*, 1925, xxxvii, 400.

SCIENTIFIC PROCEEDINGS.

New York Meeting.

Columbia University, May 18, 1927.

3541

Effect of Organ Extracts on Blood Regeneration.*

ANDREW JENEY AND JAMES W. JOBLING.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

We wish to report the results of some experiments made to determine the effects of various organ extracts on blood regeneration in animals made anemic by bleeding. This work was begun three years ago, but the contradictory findings at different times made it seem unwise to make an earlier report.

The preparations used consisted of (1) 75 per cent alcoholic extracts of the stomach, liver and spleens of calves; (2) saline suspensions of the cells of the same organs of rabbits; (3) hemoglobin solution; (4) fat-soluble non-saponifiable material from calves' livers; (5) saline extracts of these organs of rabbits which had been made anemic by bleeding.

Rabbits were used in all the experiments. They were made anemic by bleeding from the heart. The blood removed constituted about 3 per cent of the body weight, about 60 per cent of the total volume. Several blood counts and hemoglobin determinations were made before the bleeding and at frequent intervals afterwards. Our interpretation of the results of the injections of the different substances was based upon the rapidity with which the normal count was restored, as compared to a series of similar untreated anemic animals.

The results obtained with these various extracts were not very promising. At times we felt that the liver extracts were active in

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* This work was aided by a grant from the Eli Lilly Company.

stimulating blood regeneration, but subsequent experiments made it evident that we were not justified in this assumption. Our most positive findings were with the alcoholic spleen extracts. In every instance this extract prevented blood regeneration and if the injections were continued the animal died.

Our results are particularly interesting in view of those obtained with the feeding of liver by Whipple[1] in the experimental anemias of dogs, and by Minot and Murphy[2] in pernicious anemia. Several explanations of the discrepancies are possible. First, rabbits may not respond to the liver treatment; second, the active substance may have been destroyed by the heat used in drying our alcoholic extracts, though it did not exceed 45° C., or it may have become oxidized; third, our preparations were given by subcutaneous injection, theirs by mouth.

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[1] Robsheit-Robbins, and Whipple, G. H., *Am. J. Physiol.*, 1925, lxxii, 408.

[2] Minot, G. R., and Murphy, W. P., *J. Am. Med. Assn.*, 1926, lxxxvii 470.

3542

Effects of Repeated Injections of Toxins of Intestinal Bacteria on Blood Regeneration in Rabbits.*

PASTOR R. SAPINOSO AND JAMES W. JOBLING.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

There is doubt as to how much significance should be attached to the belief that certain pathological conditions are due to the absorption of intestinal toxins. Similar doubts are expressed concerning the value of interpretations based on the experimental work done, since most of it has been done with either a pure culture of bacterium isolated from the intestinal tract, or with extracts of the feces. It seems possible that mixtures of bacteria grown in the presence of protein or carbohydrates may produce toxic substances not to be found in pure cultures, and it is also possible that some unknown organism may grow under such symbiotic conditions and produce its specific toxin. The following experiments were conducted to determine the effect of toxins of the intestinal bacteria on blood regeneration.

The culture medium consisted of bouillon containing either finely

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* This work was aided by a grant from the Eli Lilly Company.

chopped beef, or brain. To half of each series was added 1 per cent soluble starch. One half of the flasks were inoculated with saline emulsions of feces from a patient with pernicious anemia, and the other half with feces from a normal individual. Control uninoculated flasks were incubated for the same periods. The flasks were removed from the incubator at intervals of 1 week, 2 weeks and 4 weeks, and the contents passed through Berkefeld filters. Only those filtrates found to be sterile by aerobic and anaerobic methods were used. The filtrates were kept in the ice-box, but even then the toxicity soon decreased.

Rabbits were used in all experiments. The filtrates were given intravenously in amounts of 0.25 to 0.5 cc., diluted 1 to 3 with salt solution, or subcutaneously in 1 cc. doses. The injections were given at intervals of 48 hours. Blood examinations were made at frequent intervals both before and after the injections were started.

All the animals developed an anemia of the secondary type, beginning after the third or fourth injection. The lowest count observed was 1,530,000. At times remissions occurred in spite of the continued injections, but they were usually of brief duration. Only an occasional nucleated red cell was observed. At times the reticulated cells were slightly increased, but for the most part they remained within the normal limits. The white count remained low. Very little, if any, change was observed in the size and shape of the red cells. Practically no immunity was produced, as doubling the dose of toxin would frequently kill animals which had received many previous injections.

3543

Effect of Bile, Sodium Salts of Bile Acids, and Unsaturated Fatty Acids on Bacteriophagic Action.

GORDON M. KLINE. (Introduced by A. B. Wadsworth.)

From the Division of Laboratories and Research, New York State Department of Health, Albany.

Previous investigators record different observations on the effect of bile on bacteriophagic action. Hauduroy,[1] Jong and Hauduroy,[2] and d'Herelle[3] found that bile was decidedly inhibitory, whereas Caublot,[4] in observations extending over a period of two years, obtained no evidence of an inhibitory action of bile on bacteriophage.

In the studies, recorded here, of the action of bile on bacteriophage, *B. coli, B. typhosus* and *B. dysenteriae* Shiga and Mt. Desert strains were used with bacteriophages obtained from stool filtrates and from culture filtrates. In no case was there any inhibition of the bacteriophage in the presence of bile, although the bile was added to broth medium in concentrations of 10 per cent and 50 per cent. Filtered bile was tested as well as bile sterilized by heat, and a peptone medium was substituted for beef infusion broth, but the end result was always the same, the bile never inhibited the bacteriophage.

Bachmann and Aquino[5] report that, in their experiments, bile stimulated the production of a lytic substance by bacteria; but these authors also obtained a transmissible lytic substance from the bacterial culture alone. With the purpose of studying this property of bile, experiments were carried out both with bacterial strains from which lytic substance had never been obtained, and with strains which were known to produce bacteriophage at frequent intervals. Bile, however, did not promote formation of bacteriophage in any of these bacterial cultures.

The effect of sodium oleate, sodium taurocholate, sodium ricinoleate and the sodium salts of the unsaturated acids of linseed oil on bacteriophagic action was studied with the same bacterial strains as those used in the bile experiments. Sodium oleate and sodium taurocholate did not inhibit in concentrations of 0.1 per cent and 0.4 per cent; on the other hand, sodium ricinoleate and the sodium salts of the unsaturated linseed oil acids showed marked inhibitory properties on the bacteriophagic action. Sodium ricinoleate was markedly inhibitive at 0.1 per cent and 0.4 per cent concentrations; the unsaturated salts from linseed oil were almost completely inhibitive at 0.1 per cent concentration and were completely so at 0.4 per cent concentration. Broth, containing these salts, inoculated with organisms without the addition of bacteriophage, yielded a good growth.

Further studies are in progress to determine the nature of the inhibition, whether the salts act by lowering the surface tension, or whether they are effective because of the unsaturated group present.

This is a preliminary report.

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[1] Hauduroy, Paul, *Comptes rendus Soc. de Biol.*, 1925, xcii, 1442.

[2] De Jong, S. I., and Hauduroy, P., *Bull. et Mém. Soc. Méd. d. Hôp. de Paris*, 1925, xlix, 1561.

[3] D'Herelle, F., The Bacteriophage and its behavior. The Williams & Wilkins Company, Baltimore, 1926.

[4] Caublot, Paul, *Comptes rendus Soc. de Biol.*, 1925, xciii, 1583.

[5] Bachmann, A., and Aquino, L.-I., *Comptes rendus de Biol.*, 1922, lxxxvi, 1108.

Secretary's Report—April 1, 1926-March 31, 1927

The large number of replies to the Secretary's letter indicated a very general willingness to cooperate. Many showed gross misunderstanding or misinformation. The following concise statement may help members to understand and through such understanding cooperate more effectively.

(1) NEW MEMBERS. *Members' dues will be $5.50, beginning October, 1927. They receive two pages free, which cost the Society $8.50. Not all members use this space. Nearly all new members do use this free space. A large increase in new members is not a source of net income, rather the reverse.*

(2) COST OF PRINTING. *We have bids from a number of responsible printers recommended by members and by editors of other journals. Our printer is $400 to $1000 lower. A change of printer is no solution.*

(3) LENGTH OF ARTICLES. *Articles over two pages are not an expense to the Society. All such space is paid for by authors.*

(4) ADVERTISING. *This is under consideration. Our best advice is that the net return will be negligible.*

(5) EUROPEAN SUBSCRIPTIONS. *Measures have already been taken to increase such subscriptions. Detailed advice is asked.*

(6) LARGE ENDOWMENT. *A committee is now negotiating for financial aid either by increasing substantially our present Endowment Fund, or underwriting the annual deficit. It may take years before final reports can be made.*

(7) SUBSCRIPTIONS FROM NON-MEMBERS. *This is practically all profit. The response to the Secretary's request for such subscriptions has been most encouraging. Subscriptions from colleagues, libraries, medical men, seems the best procedure for the present.*

I. OFFICERS, 1927-28.

A. COUNCIL.

President: S. R. Benedict.
Vice-Pres.: Peyton Rous.
Sec.-Treas.: A. J. Goldforb.
Six Elected Councillors: W. W. Palmer, (1928), H. D. Senior (1928), Oswald F. Avery (1929), Robert Chambers (1929), A. E. Cohn (1930), Alfred F. Hess (1930).
Chairmen of Branches:
Illinois: A. B. Luckhardt.
Iowa: O. H. Plaut.
Minnesota: F. H. Scott.
Missouri: John Auer.
Pacific Coast: Wm. Ophüls.
Peking: Hsien Wu.
Southern: Irving Hardesty.
Western New York: W. R. Bloor.

B. PAST PRESIDENTS.

F. B. Wilson, Simon Flexner, F. S. Lee, T. H. Morgan, James Ewing, Graham Lusk, Wm. J. Gies, Gary Calkins, George B. Wallace, Holmes C. Jackson, James W. Jobling.

Past Presidents are also members of the Council.

C. NOMINATING COMMITTEE.

A. J. Carlson, Robert Chambers, P. J. Hanzlik, H. B. Lewis, David Marine, L. B. Mendel, W. J. Mac Neal, H. C. Sherman, G. B. Wallace.

D. SECRETARIES OF BRANCHES.

Illinois: Wm. F. Petersen.
Iowa: W. W. Swingle.
Minnesota: F. H. Scott.
Missouri: P. A. Shaffer.
Pacific Coast: T. D. Beckwith.
Peking: R. H. P. Sia.
Southern: Henry Laurens.
Western New York: W. M. Sperry.

II. MEMBERSHIP AND SUBSCRIPTIONS.

Members, March 31, 1926	779	
Members elected to March 31, 1927	74	
Members reinstated	2	
Total	—	855
Honorary members March 31, 1926	3	
Honorary members elected to March 31, 1927	5	
	—	8
Total		868
Resignations year ending March 31, 1927	3	
Deaths, year ending March 31, 1927	6	
Dropped for arrears, year ending March 31, 1927	3	
Total	—	12
Net membership		851

Membership applications are first submitted to Branch officers for their approval, then referred to Membership Committee of the Society, and finally acted on by the Council.

SUBSCRIPTIONS.

Subscriptions, March 31, 1926	179	
Subscriptions, March 31, 1927		214
Number Associates subscribing, March 31, 1926	4	
New Associates subscribing to March 31, 1927	4	
Total	—	8
Free subscriptions, March 31, 1926	42	
Free subscriptions, March 31, 1927		31*
Exchanges, March 31, 1926	6	
Exchanges, March 31, 1927		18*

*Council has voted to reduce to minimum free subscriptions and exchanges to take effect October, 1927.

Total number PROCEEDINGS distributed March 31, 1926	1009
Total number PROCEEDINGS distributed March 31, 1927	1122

III. REPORT ON BRANCHES.

ILLINOIS BRANCH.

Number of members—61.

Meetings.

University of Chicago, October 27, 1926.
City Club, Chicago, December 20, 1926.
University of Chicago, March 1, 1927.
Northwestern University Medical School, May 10, 1927.

IOWA BRANCH.

Number of members—18.

Meetings.

State University of Iowa, November 3, 1926.
State University of Iowa, February 2, 1927.
State University of Iowa, April 28, 1927.

MINNESOTA BRANCH.

Number of members—40.

Meetings.

University of Minnesota, December 15, 1926.
University of Minnesota, January 26, 1927.
University of Minnesota, February 23, 1927.
University of Minnesota, May 25, 1927.

MISSOURI BRANCH.

Number of members—36.

Meetings.

Washington University, November 3, 1926.
St. Louis University, December 15, 1926.
Washington University, February 9, 1927.
St. Louis University, March 30, 1927.
Washington University, May 18, 1927.

NEW YORK.

Number of members, including foreign members—539.

Meetings.

Post Graduate Hospital, October 20, 1926.
Rockefeller Institute, November 17, 1926.
College of Physicians and Surgeons, December 15, 1926.
Cornell University Medical College, January 19, 1927.
Presbyterian Hospital, February 16, 1927.
New York University and Bellevue Medical College, March 16, 1927.
College of the City of New York, April 20, 1927.
Columbia University, May 18, 1927.

PACIFIC COAST BRANCH.

Number of members—55.

Meetings.

University of California, October 20, 1926.
Lane Hospital, December 15, 1926.
University of California Hospital, February 28, 1927.
Stanford University, April 30, 1927.

PEKING (CHINA) BRANCH.

Number of members—23.

Meetings.

Peking Union Medical College, October 25, 1926.
Peking Union Medical College, January 13, 1927.
Peking Union Medical College, March 10, 1927.
Peking Union Medical College, April 28, 1927.

SOUTHERN BRANCH.

Number of members—19.

Meetings.

Tulane University, December 2, 1926.
Tulane University, February 10, 1927.
Tulane University, May 14, 1927.

WESTERN NEW YORK BRANCH.

Number of members—52.

Meetings.

Cornell University, October 16, 1926.
Clifton Springs Sanitarium, December 11, 1926.
University of Rochester, February 26, 1927,
University of Syracuse, May 9, 1927.

IV. REPORT ON PROCEEDINGS.

A. ABSTRACTING OF PROCEEDINGS.

The PROCEEDINGS are abstracted regularly in Archives of Pathology, Biological Abstracts, Chemical Abstracts, J. of Am. Med. Assn., Physiological Abstracts, and Quarterly Cumulative Index.

B. EDITORIAL POLICY.

To avoid duplicate publication of articles, each author is requested to notify the editorial office whether the essential data of his paper has been sent for publication elsewhere.

The enlarged Board of Editors must approve papers before publication.

Members are reminded that manuscripts should not exceed two printed pages, though 5 pages will be permitted. The page is now about 375 words, two pages 750 words, and 5 pages 1875 words.

To facilitate the use of the enlarging PROCEEDINGS, experiments have been made with different types of tables of contents. It would be very helpful if members would inform the Secretary which type of contents they find most useful.

The last volume of the PROCEEDINGS (Vol. XXIII) shows the *average length of papers published, including cuts and tables, was* 1.8 *pages for members and* 1.5 *pages for non-members.*

No. papers (members)	Length	Average 1.8 pages per paper
35	½ page	
137	1 page	
104	2 pages	
48	3 pages	
24	4 pages	
1	7 pages	
2	8 pages	

V. FINANCES.

The increased cost of publication is the primary cause of the deficit for the year. (See Treasurer's report.)

The following problems were before the Society: 1. The further increase in size and cost of the PROCEEDINGS. 2. The publication of a ninth number as requested and approved by the members. This involves not only the cost of publication, but increased office expense. 3. The granting of 25 free reprints to authors, as asked and approved by the members.

It should be recalled that members receive 2 free pages a year, which cost the Society $8.50, and they received eight numbers of the PROCEEDINGS.

It was necessary to increase dues for next year to meet the deficit due to the enlarging PROCEEDINGS. To meet this cost, including the ninth number and free reprints, the members voted decisively in favor of increasing dues to $5.50 and subscriptions to $6.50. The total cost will just be met by the increase in members' dues and subscriptions, *plus the interest of the Endowment Fund.* (It should be recalled that the PROCEEDINGS contains over 1000 pages, *or the equivalent of two volumes.)*

The ninth number will be issued this June without extra cost to members. The increase in dues begins October, 1927.

To meet these costs and to provide for further conservative growth the cooperation of members is sought in two ways: (1) Increase the number of subscribers to the PROCEEDINGS from general or departmental libraries, from associates or medical workers interested in research. This is of prime importance. *If members will aid in this it would be possible to meet future growth, approve applications for admissions of new branches, make further concessions to authors, and possibly reduce dues to members.* (2) Members are requested to contribute further to the Endowment Fund.

VI. GIFTS.

The following back numbers are out of stock. To meet the demand of libraries for these, the Secretary's office will be glad to receive by gift or purchase any of the following:

Vol. I, II, III, IV, XI, XII, XIII, XIX, XX, XXI, XXII No. 2.

The Secretary wishes to acknowledge, with the thanks of the Council, the receipt of back numbers by gift from Drs. F. F. Frankel, E. C. Faust, I. G. Greenwald, M. M. Brooks, H. U. Williams, O. T. Rasmussen, Johns Hopkins Hospital Library, and the John Crerar Library.

VII. BY-LAWS.

The following By-Laws were adopted by the Society:

I. *Endowment Fund.* When the Trustees will have arranged with (name of bank to be inserted here) to act as custodians of the Endowment Fund and advisors as to legal securities, as provided by the laws of the State of New York, such action will be deemed sufficient legal security as required by Article VI, Section 3 of the Constitution.

II. *Procedure for the determination of guilt under charges affecting tenure of membership.* A. Any member who may be charged formally and in writing with conduct rendering him subject to the constitutional provisions of forfeiture of, or expulsion from, membership, shall be given ample opportunity to show the Council that the said charges are unfounded.

B. If, after a full hearing, the Council finds, by a majority vote of its total membership, that a member is guilty of conduct rendering him subject to the constitutional provisions relative to forfeiture of, or expulsion from, membership, the Council shall, in the first instance, promptly make due formal announcement of such forfeiture; or, in the second instance, shall formally propose the expulsion of that member, with a statement of the reasons therefor, by a mode of procedure in accord with the general provisions of Article III, Section 4 of the Constitution, but in a manner that will adequately afford the member involved every privilege of appeal to all the members of the Society against the proposed expulsion.

VIII. MISCELLANEOUS.

The first Life Membership has been established. Dr. A. R. Moore has given $75.00 to the Society for this purpose and has designated a colleague in Austria as recipient of the PROCEEDINGS during his lifetime.

The Council wishes to record with regret the death of the following members:
J. George Adami, C. H. Bailey, E. C. Fleischer, A. W. Hewlett, John Howland, and Franz Pfaff.

Respectfully submitted,
A. J. GOLDFORB, *Secretary.*

Treasurer's Report—April 1, 1926 to March 31, 1927

RECEIPTS.

Income, 1926-27—		
Dues	$3305.84	
Excess Space	1518.92	
Reprints	2039.82	
Authors' Changes	64.45	
Subscriptions	1228.29	
Sale of Back Numbers	377.83	
Cuts	308.10	
Bank interest	32.39	
Miscellaneous income	4.95	
Total annual income		$ 8,880.59
Special Receipts—		
Life subscription	$ 75.00	
Special deposit account, Peking Union Med. Coll.	100.00	
Contributions to Endowment Fund	326.00	
Total special receipts		501.00
Cash balance, April 1, 1926		2,136.98
Total cash available, April 1, 1926, to April 1, 1927		$11,518.57

DISBURSEMENTS.

Publication Costs of PROCEEDINGS—		
Printing	$4113.79	
Reprints	2134.96	
Cuts	448.60	
Total publication cost		$ 6,697.35
Administrative Expense—		
Office supplies (printing, paper, etc.)	424.02	
Salaries	1907.40	
Postage	140.24	
Telegrams	3.25	
Storage and Insurance	79.46	
Miscellaneous	22.60	
Purchase of back numbers	44.35	
Refunds	41.57	2,662.89
Total annual disbursements		$ 9,360.24
Transferred to Endowment Fund		534.35
Cash balance, April 1, 1927		1,623.98
Total cash available during 1926-27		$11,518.57

SUMMARY.

Disbursements (net)	$9360.24	
Income (net)	8880.59	
Deficit for year		$ 479.63
Bills payable	none	
Bills collectable, April 1, 1927	$1125.62	
Bills collectable, April 1, 1926	1051.17	
Bills collectable in excess of last year		$ 74.45
Net deficit for year		$ 405.18

ENDOWMENT FUND.

In R.R. Coop. Bldg. Loan Assn., April 1, 1926	$8774.08
Contributions to April 1, 1927	326.00
Returned interest of last year	208.35
Interest for year, 4/1/26 to 4/1/27	444.63
Total in R.R. Coop. Bldg. Loan Assn., 4/1/1927	$9753.06

3544

The Structure of B. Anthracis and the Reversal of the Gram Reaction.

JOHN W. CHURCHMAN.

From the Laboratory of Experimental Therapeutics, Cornell University Medical School.

If a small amount of aqueous solution of acriviolet (1 oeseful of a 1 per cent solution) be added to ½ cc. of a heavy aqueous suspension of *B. anthracis* it will be found that the organisms are changed by the exposure from sharply Gram positive to sharply Gram negative.* The speed with which this change takes place varies with the strain. In one of the 7 strains examined the change was complete in 2 hours. In other strains it was complete only at the end of 19 hours. Exposure to gentian violet gave results similar to those obtained with acriviolet.

The Gram negative forms present after exposure to these dyes are notably smaller in caliber than the Gram positive forms. Measurements of the stained specimens with a filar micrometer show the loss of caliber to be on the average in the neighborhood of 25 per cent. This measured difference in size in the stained specimens is probably in part due to the piling up of stain on the Gram positive segments since the difference in size, though demonstrable in unstained hanging drop specimens, is of lesser degree than in the stained specimens.

The change in Gram reaction and the change in size cause a striking picture in smears, stained by Gram's method, made from suspensions to which dye has been added, and which are examined at a period when the changes produced are as yet only partial. Chains of bacteria are seen in which stout, deeply Gram positive segments alternate with slender, sharply Gram negative segments. When individual bacteria are present instead of chains, the picture is that of a smear of two different organisms, one stout and Gram positive, the other slender and Gram negative.

A change similar to that produced in *B. anthracis* is also produced by these dyes in most of the other spore bearing aerobes (23 strains examined). The results, however, with some of these strains were not quite so striking nor so constant as in the case of *B. anthracis.* In contrast to the behavior of the spore bearing aerobes the majority of Gram positive non-spore bearers (notably, the cocci) when exposed to these dyes remain unchanged; they are as sharply Gram positive at the end of the experiment as at the beginning. About fifty strains of Gram positive non-spore bearers were examined and the majority followed the rule just stated. There were, however, exceptions, notably the pneumococci which proved too fragile for this type of experiment and about which no definite statement can be made.

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* Burke's modification of the Gram method was used throughout these experiments.

If, therefore, to a mixed suspension containing two sharply Gram positive organisms like *B. anthracis* and *micrococcus freudenreichii* (both of which follow the rule stated above) acriviolet be added and smears, stained by Gram, be examined at the end of 24 hours a striking contrast appears: all, or nearly all, the individuals of *B. anthracis* have become Gram negative while all, or nearly all, the individuals of *M. freudenreichii* remain Gram positive. It is clear from this experiment that the Gram positivity of these two Gram positive organisms is very different in character; in the case of *B. anthracis* it is a surface characteristic rather easily destroyed, the center of the organism being sharply Gram negative.

The phenomena described are due to the facts that *B. anthracis* consists of two distinct parts, a Gram positive cortex and a Gram negative medulla,† and that the cortex may be removed. It may be removed by exposure to acriviolet or gentian violet. It may also be removed, though with much less certainty, by hydrolysis in distilled water. It doubtless sometimes disappears of itself, a fact which accounts for the Gram negative forms (always slender) occasionally seen in smears of *B. anthracis* made from cultures. The existence of a Gram positive cortex and a Gram negative medulla may be beautifully demonstrated by staining smears from a young (4 hr.) culture of *B. anthracis for 5 seconds* with Burke's gentian violet, fixing for *5 seconds* with Burke's iodine, decolorizing for *5 minutes* with acetone ether, and counterstaining with safranin. By this method of rapid staining and intensive decolorization the Gram positive material is partially or completely removed from the cortex and bacteria are seen in all stages of decolorization. They appear either as entirely Gram negative forms, or as sharply Gram negative rods to the surface of which are attached lumps or plaques or dots of undissolved Gram positive material, between which the pink safranin-stained central rod is plainly seen.

That the difference in size between the Gram negative forms resulting from exposure to the dye, and the Gram positive forms in the controls is actually due to a loss of substance seems likely, though perhaps not definitely proven, from the results of experiments in which the bacterial bodies were weighed before and after exposure to the dye. That the material lost is protein, or at least ammoniacal in character, is proven by the positive ninhydrin tests obtained with the Berkefeld filtrate of a suspension of *B. anthracis* which had been exposed to gentian violet.

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† "Medulla" and "cortex" are used tentatively as convenient descriptive terms; "ectoplasm" and "endoplasm" might be better.

3545

Reciprocal Influence of Concomitant Infections: Syphilis and Vaccinia.

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The influence which one infection may have upon the manifestations of another has been investigated in a number of conditions in connection with a general study of the factors concerned in determining the course and outcome of disease processes. In the case of syphilis and vaccinia of the rabbit, the result of simultaneous inoculations of *Tr. pallidum* and vaccine virus has been studied. Other experiments have dealt with the syphilitic reaction in rabbits immune to vaccine virus, and conversely, with the vaccine reaction in rabbits infected with *Tr. pallidum.*

The Nichol's strain of *Tr. pallidum* and the Noguchi strain of vaccine virus were used; groups of 5 or 10 male rabbits were employed for each series. The syphilitic tissue emulsion was injected in one testicle or intracutaneously on the sheath; the inoculation of vaccine virus was made on the shaved skin of the body by rubbing the infected tissue emulsion into scarified areas, and by intracutaneous injection. In other groups, the syphilitic and vaccine virus emulsions were both injected in the same testicle. Control series of rabbits were inoculated with each of these materials.

The observation period varied from 3 to 5 months. An essential requirement of the work was the frequent examination of the rabbits in order that the syphilitic process in the several groups could be compared. The features of this infection which are especially important for such comparisons are illustrated by the following examples: the incubation period, character and duration of the primary and metastatic orchitis, the occurrence of scrotal edema, the time of development of generalized lesions, their distribution, number and duration, and the initiation of latency, that is when all manifestations of the disease have healed.

The results of completed experiments and the observations of others still in progress may be summarized as follows:

The character of the syphilitic infection has been markedly affected by the simultaneous inoculation of vaccine virus. With the intracutaneous injection of vaccine virus, the severity of the syphilitic process was greatly increased, as shown in the case of both primary and secondary lesions, but particularly the latter in which an increased distribution, greater size, longer duration and in some series increased incidence, were especially marked. On the other hand, the syphilitic infection which developed after the combined injection of vaccine virus and syphilitic material in the same testicle was modified in the direction of decreased severity. The mildness of the disease in these rabbits as compared with the controls was very striking. An immunity to vaccine virus present at the time of syphilitic inoculation was also associated with alterations in the manifestations of the infection, and in general, the disease was less severe than in the controls. The reaction to vaccine virus under the condition of simultaneous inoculation with syphilitic material will be reported later, but in general, it may be said that it is accelerated and exaggerated. This was also the case in many instances in which syphilitic rabbits were vaccinated.

The interpretation of these results will not be attempted here, but it is obvious that as far as the syphilitic process is concerned, their significance is intimately related to the factors of host susceptibility and resistance as determinants of the course and outcome of this particular infection.

This is a preliminary report.

3546

A New Dietary Deficiency With Highly Purified Diets.

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In the course of our work on vitamine E it became necessary some years ago to attempt to withdraw all traces of this substance from dietaries. But various workers, including ourselves, have reported inferior animals when ordinary casein is extracted for a long time with solvents for E—alcohol and ether. This effect could be due to removing impurities or to lowering the nutritive value of the protein itself. The solution of this problem is obviously facilitated by purity and hence constancy in the remaining ingredients of the diet. It is established that the rat can use without embarrassment either fat or carbohydrate alone for the complete satisfaction of its caloric needs. The use of starch as a source of energy involves the inclusion of many impurities. The recent papers of Taylor and Iddles,[1] for example, show that cornstarch contains about 0.6 per cent of fatty substance, which is within the granule and is non-extractable. The use of sucrose has been decided upon after a comparison of this sugar with chemically pure glucose and chemically pure lactose. The former offers no advantage and lactose proved entirely unsuitable, since the animals soon declined and died apparently because of intestinal disturbances. The sucrose is made by recrystallizing the best grade of commercial cane sugar from 80 per cent alcohol. The casein is prepared according to Van Slyke, and is a dry white powder which has no ether extractable material and an ash content of not over 0.2 per cent. The salts are of the highest purity obtainable in the market. The pure diet employing these substances (our Diet 519) is as follows:

Casein, Van Slyke	50
Sucrose, pure	150
Salts (185)	8

KI added to water.
Distilled water *ad libitum*.
Cod liver oil 2 to 3 drops daily.
Yeast 700 to 1000 milligrams daily.

The animals are taken at 21 days of age and kept in individual metabolism cages so there is no access to feces or urine. They are kept in a large, light, well ventilated metabolism room whose temperature is maintained between 22° C. and 25° C. by thermostatic control. All vitamine doses are fed in a separate dish and are quickly consumed.

The results summarized here have been secured during the past twelve months but the groups were small and the data must be considered as preliminary in nature. When the pure diet was fed to twelve females with 700 milligrams of whole dried yeast (Fleischman) and 3 drops of cod liver oil (Patch) daily, they plateaued when about 125 grams in weight. This weight was attained slowly rather than rapidly and ovulation did not occur during the first four months of life. (Normally, ovulation begins before the fiftieth day of life.)

When the pure diet was fed to males and supplemented by 750 milligrams of yeast (extracted with pure absolute ether) and 2 drops of cod liver oil, the males grew at the apparently normal rate of 2.7 grams daily for the first 18 days, reached about 100 grams in weight and quickly plateaued. No further gain was observed in four weeks. Males fed 750 milligrams of fresh wheat germ daily instead of the yeast have reached the same weight but much more slowly.

The most recent data show that females which receive 600 milligrams of fresh wheat germ daily (instead of yeast) plus 3 drops of cod liver oil gain less than 1.5 grams daily during the first 30 days on the diet and they plateau at about 100 grams. Females receiving 1.0 grams of ether extracted yeast daily plateau at about 120 grams.

In examining these diets for any known deficiency we feel that vitamines A, D, and E can be ruled out at once. We have often tested our supply of Patch's cod liver oil and find that in vitamine A experiments (irradiated lard) 2 milligrams daily allows resumption of growth by rats. We feed either 40 or 60 milligrams daily in this diet. We know that 20 milligrams daily is an ample supplement to our ordinary casein and cornstarch diets. The use of wheat germ for B in some of the experiments supplied an overabundance of E.

There is more confusion concerning the growth vitamine, B. Osborne and Mendel[2] have found that normal growth to 100 grams of weight is given by 100 milligrams of Harris yeast, and normal growth to 300 grams is given by 200 milligrams of Harris yeast. We have found that when wire bottoms are used 200 milligrams of Harris yeast will allow females to grow only to 150 grams of weight and maintains them at this level. Furthermore, during the period of most rapid growth their daily gain is about 1.5 gram instead of 2.5 to 3.0 grams. Smith, Cowgill and Crall[3] have found that the yeast dose must be increased about 50 per cent when wire bottoms are used. *But when using the pure diet we find no growth above* 100 *grams weight when* 600 *to* 750 *milligrams of wheat germ or yeast are used,* and grave subnormality is detected in animals receiving even 1 gram of yeast daily. It would appear that we must radically revise our conception of the amount of vitamins B present in the usual simplified diets if this is the limiting factor in our pure food experiments. The complicity of B is highly improbable. It is more likely that the trouble has resulted from the purification of the main dietary ingredients. It is difficult to believe that the purification has caused any impairment in the nutritive value, as ordinarily understood, of either the sugar or casein. A restitution can be effected from the above dwarfism when small amounts of 2 natural substances are added to the rigorously purified diet. Females receiving 800 milligrams of yeast plus 10 grams of fresh lettuce leaf, or 0.5 grams of fresh beef liver are equalling in growth the best animals on natural foods. The pure diet when supplemented by these amounts of lettuce or liver also allows gestation and the birth of good litters. Lactation is deficient but these data are still too scanty for summation of our experience here. The simplicity and purity of this basal ration makes its value for nutrition research evident. It perhaps approaches in purity the culture media used by plant physiologists who have recently made remarkable findings concerning the mineral requirements of plants.

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[1] Taylor and Iddles, *J. Ind. and Eng. Chem.*, 1926, xviii, 713.

[2] Osborne and Mendell, *J. Biol. Chem.*, 1922, lix, 739.

[3] Smith, Cowgill and Crall, *J. Biol. Chem.*, 1925, lxvi, 15.

3547

Observations on the Filtrability of B. Tuberculosis.

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A series of 21 guinea pigs was inoculated with the Berkefeld filtrates of tuberculous material. Of 12 animals autopsied so far, acid-fast bacilli have been identified in the direct smears from the lung, inguinal and tracheo-bronchial glands of 3 of the animals. Prolonged search was necessary to demonstrate the organisms which, however, occurred usually in large clumps when found.

No definite evidence of tuberculosis was found by histological methods, except in the lungs of two of the positive animals. These showed small punctate areas of granulomatous, endotheloid hyperplasia, not specific for tuberculosis. The lesions did not differ materially from those found in the lungs of one out of twelve control animals examined.

Exhaustive search of the smears of the lymph glands and lungs of 12 uninoculated animals did not disclose the presence of any acid-fast bacilli. Cultures and inoculation experiments from positive animals yielded negative results.

These results as a whole appear in accord with those of the French school, who believe they have demonstrated the filtrability of this organism. Lacking as they do cultivability, or the power of producing classical tuberculosis, we feel that the nature of the acid-fast organism in the smears is uncertain as yet.

On the other hand, one of the animals inoculated with the filtrate under different conditions than the rest died between the 6th and 7th month of classical tuberculosis. The lesions involving the abdominal lymph glands, lungs and liver were widespread and unmistakable. Acid-fast bacilli were readily found and on injection again produced classical tuberculosis. A control animal inoculated with the same filtrate as the test animal but under the usual conditions died in five weeks of pneumonia but showed no evidence of tuberculosis. Attempts to repeat this rather noteworthy result are under way.

This is a preliminary report.

3548

Peptone Hypoglycemia.

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The effect of peptone on blood sugar has been determined by several investigators, but the results obtained have been discordant. Henderson and Underhill[1] in 1911, and Kuriyama[2] in 1917 reported hyperglycemia after intravenous injection of peptone. On the other hand, McGuigan and Ross[3] in 1915 found marked hypoglycemia, while Menten and Manning[4] in 1927 observed hyperglycemia with three samples of Witte's peptone, hypoglycemia with one sample, and no change in blood sugar with another sample.

The experiments to be described were made some time ago, but are now reported in view of Menten and Manning's paper. Our results throw light on the possible cause of the variations in blood sugar obtained after administration of peptone. The experiments recorded differ from those of previous investigators. The diet of the test rabbits was noted and kept constant in view of the relation of food to the glycogen content of the liver and to the blood sugar level. The rations consisted of alfalfa and oats. The animals were deprived of food but not of water, 24 hours previous to injection. The injections were made subcutaneously. The material was not peptone dissolved in water with saline, but an extract of peptone prepared similarly to insulin.

Twenty grams of peptone of different makes were mixed with 1000 cc. of 70 per cent alcohol containing 0.4 per cent sulphuric acid. The mixture after standing 24 hours was filtered, and the filtrate evaporated at reduced pressure until the odor of alcohol disappeared. The aqueous residue was treated with 95 per cent alcohol, and a brown gelatinous precipitate was obtained. This was separated by filtration and dissolved in a small quantity (10 to 15 cc.) of water (pH 3) acidified with hydrochloric acid. Blood sugar was determined by the method of Schaffer and Hartmann. The injection of the slightly acidified water alone increased the blood sugar but slightly.

HYPOGLYCEMIC EFFECTS OF PEPTONE.

1. Sample: Parke and Davis peptone. Weight of rabbit, 1900 grams.

	Percent of blood sugar
Normal	0.110
1 hour after injection	0.085
3 hours after injection	0.054
5 hours after injection	0.054

2. Sample: Difco peptone. Weight of rabbit, 1550 grams.

Normal	0.092
1 hour after injection	0.098
3 hours after injection	0.079
5 hours after injection	0.072

3. Sample from bottle labelled Meat Peptone. Weight of rabbit, 2250 grams.

	Percent of blood sugar
Normal	0.130
1 hour after injection	0.092
3 hours after injection	0.060
6 hours after injection	0.060

4. Sample: Merck peptone. Weight of rabbit, 1400 grams.

Normal	0.110
1 hour after injection	0.098
3 hours after injection	0.092
5 hours after injection	0.124

The general trend of our results indicates that peptone contains a hypoglycemia-producing substance, the amount of which varies markedly with the commercial source of the peptone. The substance that lowers blood sugar is not formed as a result of the acid-alcohol treatment, for McGuigan and Ross,[3] and Menten and Manning[4] have reported hypoglycemia as the result of intravenous injection of straight peptone. The work of Henderson and Underhill,[1] Kuriyama,[2] Menten and Manning[4] points also to the presence in peptone of a hyperglycemia-producing substance. The contrary findings in the literature may be expected in view of the variability in the composition of peptone. Whether a sample of peptone causes a lowering or heightening of blood sugar may depend upon the predominating presence of the substance producing hyperglycemia or hypoglycemia.

In interpreting results on blood sugar, however, we must always bear in mind the condition of the animal. A low glycogen reserve may alter very markedly the response of the rabbit to blood sugar changes. Adrenalin, which ordinarily produces hyperglycemia, may give no change in blood sugar or a hypoglycemia, depending upon the amount of glycogen in the liver. Infection also influences the blood sugar level.[5] Spontaneous infection with *Bacillus paratyphosus B* may sometimes be found.[6] Stock rabbits may develop symptoms of hypoglycemia due to liver cysts containing *Coccidia oviforme.* Degenerative changes have been observed in the liver in infected rabbits.[6, 7] This damage may affect its glycogen content as well as its function with relation to glycogenesis, glycogenolysis or gluconeogenesis. Since injections of peptone also influence the condition of the liver, it is likely that various samples of this preparation may carry different quantities of the substance producing anatomical and physiological derangement.

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[1] Henderson, Y., and Underhill, F. P., *Am. J. Physiol*, 1911, xxviii, 275.

[2] Kuriyama, *J. Biol Chem.*, 1917, xxix, 127.

[3] McGuigan, H., and Ross, E. L., *J. Biol. Chem.*, 1915, xxii, 417.

[4] Menten, M. L., and Manning, H. M., *J. Biol. Chem.*, 1927, lxxxii, 255.

[5] Zeckwer, I. T., and Godell, H. I., *J. Exp. Med.*, 1925, xlii, 43. Levine, V. E., and Kolars, J. J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 36.

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[7] Collip, J. B., *J. Biol. Chem.*, 1923, lvii, 163.

3549

Diet and Tissue Growth. VI. Relation of Age to Renal Injury on Diet Rich in Protein.

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In an attempt to explain the discrepancy between the results reported by different investigators on the relation between dietary protein and renal lesions, Moise and Smith[1] have postulated that young rats may have greater powers of adaptation than old ones, and accordingly may be less susceptible to the injurious effect of an excessively high protein ration. This suggestion was based upon the observation of Arataki,[2] that the formation of glomeruli continues throughout the first hundred days of life, and upon the fact that a very large proportion of the published observations reporting absence of pathologic renal lesions were initiated on young rats. In studies (Moise and Smith[1]) with adult rats fed adequate diets rich in protein, structural lesions were observed at intervals from 90 to 150 days after unilateral nephrectomy. These lesions included both glomerular and tubular changes. The former consisted in serum in the capsular spaces, proliferation of the epithelium of Bowman's capsule with and without adhesions between the tuft and capsule, fibrous thickening of Bowman's capsule, partial fibrosis of the glomerular tuft, and in many instances infiltration of round cells in and around these areas. The most striking tubular changes were dilatation and active proliferation of the lining epithelium. The experiments were initiated at ages between 121 and 202 days and completed at ages between 271 and 348 days.

In our further studies of a similar nature the right kidney was removed from a small series of rats at the age of 30 days. Immediately following the nephrectomy the animals were divided into two groups, placed on the "standard" diet (18 per cent casein) and high protein ration (85 per cent casein) respectively. At the present time specimens of the left kidney are available at time intervals after nephrectomy, varying from 177 to 384 days on the standard diet, and from 171 to 277 days on the high protein food. The latter group showed a very small amount of subcutaneous and intra-abdominal adipose tissue.

The left kidneys of the animals on which a right nephrectomy was performed at the age of 30 days show no changes analogous to the renal lesions observed in the rats operated on after maturity. In the "young" rats maintained on the standard diet for periods varying from 177 to 384 days after a right nephrectomy, no significant renal lesions were observed. In a single section rare minute focal accumulations of round cells was found. On the other hand, in the "young" rats maintained on the high protein ration for periods after nephrectomy varying from 171 to 277 days, focal accumulation of round cells was frequently observed. These changes are not of the same character or extent as the lesions observed in the earlier experiments initiated on adult rats, although the time interval after nephrectomy was considerably longer in the experiments with "young" animals. The changes in the "adult" group of rats resemble more closely the senescent focal lesions that are found in aged rats (Moise and Smith,[1] Reader and Drummond,[3] and Osborne, Mendel, Park and Winternitz[4]).

These observations provide additional suggestive evidence that young animals have greater powers of withstanding the injurious effect of an extremely high protein diet than do rats subjected at an older age to the same diets.

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[1] Moise, T. S., and Smith, A. H., *J. Exp. Med.*, in press.

[2] Arataki, M., *Am. J. Anat.*, 1926, xxxvi, 399.

[3] Reader, V. B., and Drummond, J. C., *J. Physiol.*, 1925, lix, 472.

[4] Osborne, T. B., Mendel, L. B., Park, E. A., and Winternitz, M. C., *J. Biol. Chem.*, 1927, lxxi, 317.

3550

Changes in Oxygen Capacity of Blood Pigment in Rabbits Following Splenectomy.

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Nine rabbits were splenectomized, the operative procedure being done as simply and rapidly as possible. Ether anesthesia was used in all cases. Initial blood samples were taken from the ear vein of each rabbit the day before operation and were analyzed for total blood pigment and oxygen capacity by the Van Slyke carbon monoxide method.[1] In all of these initial samples the agreement between the total and the oxygen carrying hemoglobin was within the limits of experimental error. A spectrophotometric analysis of each sample was also made.

The rabbits were bled from the ear daily for three, four, or five days after operation and the blood samples analyzed as before. In every instance it was found that the oxygen capacity dropped below the total pigment, in most cases showing a maximum deviation of 10 to 15 per cent of the total pigment on the second day and returning to the same value as the total hemoglobin on the third or fourth day after operation. It was first thought that the discrepancy between the oxygen carrying pigment and the total hemoglobin content might be due to the presence of methemoglobin. The results of the work with the spectrophotometer did not substantiate this idea, however, as the ratios obtained for the light absorption at two given wave lengths (A = 5400 and A = 5600) are entirely different from those obtained in solutions of known methemoglobin content. Further work is necessary before any statement can be made as to the nature of this reduced oxygen carrying power. Such work is now in progress.

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As a control a laparotomy without the removal of the spleen was performed on one rabbit. There was no discrepancy found between total and oxygen bearing pigments.

Two rabbits were not bled, except for the initial samples, until the fifth day after splenectomy. At that time both showed a definite loss in oxygen carrying hemoglobin, one of 6, the other of 12 per cent of the total pigment. Both had returned to normal relations by the twelfth day. This suggests the possibility that the stimulus of the daily bleedings, 5 to 7 cc. being taken at a time, may play some part in causing the more rapid return to normal in those rabbits that were bled frequently immediately after operation. Further experimentation is being carried on to clarify this point.

This is a preliminary report.

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[1] Van Slyke, D. D., *J. Biol. Chem.*, 1926, lxvi, 409.

3551

Gold Preparations in Therapy.

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Gold preparations have recently found clinical application in tuberculosis and certain diseases of the skin. Among these preparations are Sanocrysin or gold and sodium thiosulfate, Krysolgan and Triphal.

Sanocrysin or gold and sodium thiosulfate was originally prepared by Fordos and Géllis in 1845. It has been improved on by Mollgaard and contains about 37.5 per cent gold and 26.2 per cent sulphur, which would correspond to the formula $3Na_2S_2O_3Au_2S_2O_3$ $4H_2O$.

Triphal, one of the other preparations studied by us from a toxicological point of view, contains approximately 44 per cent gold and 7.1 per cent sulphur, nd this corresponds to the chemical formula $C_6H_3N\text{-}NH\text{-}CSAu\text{-}COONa\ 2H_2O$ or the sodium salt of Aurothiobenzimidazolcarboxylic acid.

Previous investigations[1] have shown that under some conditions, gold salts tend to produce marked kidney and liver damage as well as urticaria, pruritus and erythematous eruptions. With these possibilities in mind, the present report deals with a toxicological study of the three common gold preparations which are available.

Examination of Tables I, II and III, shows the effects of the injection of these drugs in normal Albino rats, with the action upon the lungs, liver and kidneys. The injections were carried out on normal healthy Albino rats from a common source; they were maintained on a constant, well balanced diet composed of whole wheat flour, corn meal, skim milk, calcium carbonate, and sodium chloride, together with the addition of 2 per cent of active cod liver oil.

Examination of these tables further shows definitely the marked difference in toxicity of these various preparations, and this observation alone has a distinct bearing on the clinical application of these drugs.

The clinical discussion will be found in another article.

TABLE I.

Toxicity of Krysolgan.*

Albino rats injected intravenously.

Dose mg./kg.	Rat No.	Length of life	Ending	Lungs	Liver	Kidneys	Remarks
1	1637	7 days	Killed	Normal	Normal	Normal	
1	1638	7 days	”	”	”	”	
2	1639	7 days	”	”	”	Enlarged nephrosis	Lost weight after injection
2	1640	7 days	”	”	”	”	” ”
3	1641	2 days	Died	Congested pleural fluid increased	”	Nephrosis	Hemorrhages in thymus and adrenals. Colon empty.
3	1642	3 days	”	”	Swollen and dark	”	Retroperitoneal tissue oedematous. Bladder empty and contracted.
4	1643	3 days	”	”	” ”	”	
4	1644	3 days	”	”	” ”	”	
5	1645	3 days	”	”	” ”	”	
5	1646	3 days	”	”	” ”	”	Retroperitoneal tissue oedematous.

* Krysolgan (according to Schering circular) is employed in doses of 0.0015 grain to 1.5 grains. In preparing the solution for the above Krysolgan toxicity test the contents of two 0.050 grain ampules (6.5 milligrams) were dissolved in 10 cc. freshly distilled water.

TABLE II.
Toxicity of gold and sodium thiosulphate.
Albino rats injected intravenously.

Dose mg./kg.	Rat No.	Length of life	Ending	Lungs	Liver	Kidneys	Remarks
40	1648	7 days	Killed	Normal	Normal	Normal	
40	1649	7 days	"	"	"	"	
60	1650	7 days	"	"	"	Enlarged nephrosis	Lost weight after injection
60	1651	7 days	"	"	"	" "	" "
80	1652	3 days	Died	"	"	" "	" "
80	1653	4 days	"	"	"	" "	" "
100	1654	2 days	"	"	Swollen and dark	" "	Small intestines congested. Colon empty.
100	1655	5 days	"	"	Normal	" "	
120	1656	4 days	"	"	"	" "	
120	1657	4 days	"	"	"	" "	

TABLE III.
Toxicity of Triphal.
Albino rats injected intravenously.

Dose mg./kg.	Rat No.	Length of life	Ending	Lungs	Liver	Kidneys	Remarks
80	1599	7 days	Killed	Normal	Normal	Normal	Gained in wgt. after injection.
80	1602	7 days	"	"	"	"	" "
80	1605	7 days	"	"	"	"	" "
100	1600	7 days	"	"	"	"	" "
100	1603	7 days	"	"	"	"	" "
100	1606	7 days	"	"	"	"	" "
120	1601	7 days	"	"	"	"	" "
120	1604	7 days	"	"	"	Swollen nephrosis	" "
120	1607	7 days	"	"	"	"	" "
140	1608	3 days	Died	"	"	Slight nephrosis	Colon empty.
140	1609	3 days	"	"	"	"	Colon contained well formed feces.

It might be stated at this time that the use of Triphal clinically, shows a cure in 77.2 per cent of the cases treated with Triphal as compared with about 30 per cent cures with the use of gold and sodium thiosulfate. These toxicity studies reported in Tables I, II and III, also indicate the importance of the removal of focal infections before the injection of gold salts of this nature. It will be readily seen from the toxicity table on Triphal that this drug, from a toxicological point of view, will serve as a safe effective means of application to the treatment of *Lupus erythematosus.*

These studies were carried out entirely with the idea of application to the treatment of skin diseases such as *Lupus erythematosus, Lupus vulgaris* and the tuberculids.

Of the three preparations examined, Triphal shows a much lower toxicity, greater stability in powder form and also in solution. Solutions of gold and sodium thiosulfate decompose readily, yielding a white precipitate and giving a marked odor of hydrogen sulfide.

This is a preliminary report.

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3552

Carbon Dioxide and Bacterial Toxin Production: Preliminary Report on Diphtheria Toxin.

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Considerable difficulty is still being experienced in obtaining uniformly potent toxins, even under the best of known conditions. The purpose of this investigation has been to determine whether carbon dioxide bears any definite relationship to toxin production.

The commonly used medium containing meat infusion, 2 per cent Difco Proteose Peptone and 0.5 per cent NaCl was employed. The reaction was adjusted to pH 7.5. After clarification by heat, the filtered medium was distributed in 500 cc. Erlenmeyer flasks, 90 cc. to each flask. Final sterilization was accomplished by autoclaving at fifteen pounds pressure for fifteen minutes.

Immediately after cooling each flask was inoculated with 1 cc. of a 24 hour broth culture of *C. diphtheriae* (Park No. 8).

The inoculated flasks were divided into 4 sets, 4 flasks in each set, and incubated under the following conditions: 1 was incubated under ordinary conditions. 2 was aerated with air. 3 was aerated with carbon dioxide free air. 4 was aerated with an atmosphere containing 3 per cent carbon dioxide.

All aerated flasks were aerated at the rate of 100 cc. per hour, and incubation was at 37° C.

One flask was removed from each set at intervals of three, five, eight and 20 days from the time of inoculation, and the following determinations made on each culture: (1) pH at time of harvesting, (2) mg. of ammonia per 100 cc. of culture, (3) amount of sediment per 100 cc. of culture (estimated by centrifuging an aliquot portion in a calibrated centrifuge tube), (4) number of viable organisms per cc., as determined by the plating method, and (5) the toxicity, which was determined by the intracutaneous method originated by Römer and Sames[6] and later developed and applied by Glenny and Allen.[4] The results were recorded in Ln/500 doses, a dose in this case being the minimum amount of toxin which when injected intracutaneously together with 1/500 of a unit of diphtheria antitoxin causes a small area of necrosis distinguishable five days after the injection.

The results of the first experiment are recorded in Table I.

TABLE I.

Culture.	No. of days incubation	$\frac{Ln}{500}$ dose	pH when harvested	Increase in mg. NH_3-N per 100 cc. culture	Sediment per 100 cc. culture	No. of viable org. per cc. of culture
Set No. 1. (Incubated under ordinary conditions)	3	>.01	8.6	20.8	1.1 cc.	250,000,000
	5	.003	8.8	19.2	1.2 cc.	325,000
	8	.0018	8.9	16.3	1.1 cc.	2,800
	20	.005	8.9	6.4	.8 cc.	60,000
Set No. 2. (Aerated with normal air)	3	>.01	8.4	20.7	1.2 cc.	63,000,000
	5	>.01	9.0	22.0	1.2 cc.	26,000
	8	.004	8.9	21.0	1.1 cc.	3,000
	20	.008	9.0	19.0	1.1 cc.	8,000
Set No. 3. (Aerated with CO_2-free air)	3	>.01	8.4	21.7	1.2 cc.	360,000,000
	5	.0025	8.8	22.0	1.2 cc.	257,000
	8	.0025	9.0	21.0	1.0 cc.	2,000
	20	.005	9.0	20.0	1.0 cc.	1,000
Set No. 4. (Aerated with air plus 3% CO_2)	3	>.01	7.8	22.0	1.2 cc.	315,000,000
	5	.0008	7.8	23.7	1.2 cc.	227,000,000
	8	.0008	8.0	24.1	1.2 cc.	95,000,000
	20	.001	8.0	24.7	1.2 cc.	13,000,000

Results with Difco-Proteose Peptone.
> = Greater than.

All flasks were kept tightly stoppered after removal from the incubator, to prevent the escape of carbon dioxide. The amino acid content of each culture was determined by means of the Sörenson Formol Titration, but no marked differences were obtained between the aerated cultures and those which were not aerated. In both cases there was a slight drop in amino acid content during the first few days of incubation, which was followed by an increase. Surface tension determinations were made on each culture, by means of the DuNöuy Tensiometer. No marked changes in surface tension resulted in any of the cultures.

The ammonia content of all cultures showed a sharp increase during the first three days of incubation. In the case of the cultures grown under ordinary conditions this increase was followed by a gradual decline, as compared to a continuous increase in the flasks aerated with 3 per cent carbon dioxide. Apparently the ammonia content of cultures incubated under carbon dioxide is an indication of the pronounced peptolytic activity of the diphtheria organism.

A number of investigators, including Kligler[5] and Bunker,[3] have suggested that the need of "peptones" of high proteose content in toxin production is possibly due to their buffering action. By aerating the culture flasks with 3 per cent carbon dioxide it is possible to control the reaction of the medium, as shown in Table I.

Experiment No. 1 was repeated, using Difco-Bacto Peptone, which is known to be relatively low in proteose content, in place of Difco-Proteose Peptone. The results are given in Table II.

TABLE II.

Culture	No. of days incubation	$\frac{Ln}{500}$ dose	pH when harvested	Increase in mg. NH_3-N per 100 cc. culture	Sediment per 100 cc. culture	No. of viable orgs. per cc. culture
Ordinary conditions	8	>.01	9.0	12.6	.8 cc.	473,000
Aerated with air	8	>.01	9.0	13.0	.8 cc.	5,000
Aerated with air plus 3% CO_2	8	>.01	7.8	17.3	1.0 cc.	25,000,000

Results with Difco-Bacto Peptone.

These results show that little, if any, toxin was obtained with this peptone, even though the reaction was kept close to the initial pH. It would seem that the proteose fractions of "peptones" are necessary for toxin production for other reasons than their buffering action.

Apt and Loiseau,[2] Apt,[1] and Wolf[7] have found that the diphtheria bacillus, when grown in sugar-free peptone meat infusion broth, produces considerable amounts of carbon dioxide by the destruction of amino-acids, enough to have a marked effect on the reaction of the culture.

The results presented in this paper show that the carbon dioxide content of diphtheria cultures has considerable influence on the amount of toxin present when the cultures are harvested. In cases where large numbers of cultures are grown in an incubator with poor ventilation, enough carbon dioxide may accumulate to prevent the deterioration of the toxin, once it is formed. Where few cultures are grown in incubators with better ventilation, the carbon dioxide readily diffuses out of the medium, resulting in a more rapid rise in pH and more rapid deterioration of the toxin. The obtaining of a more potent toxin and a lower pH where flasks containing a liter or more of culture are used, as compared with small flasks containing 100 cubic centimeters or less, may be explained as a rule in a similar way.

CONCLUSIONS.

1. Carbon dioxide plays an important rôle in toxin production, by preventing the destruction of the toxin, once it is formed. This is probably done by regulating the reaction of the medium.

2. It is possible to control the reaction of *C. diphtheriae cultures* of an initial pH 7.4 to 7.6 by passing an atmosphere containing from 3 to 5 per cent CO_2 over them. By this procedure an abundant supply of oxygen is maintained.

3. Increased CO_2 tension also prevents the rapid destruction of the organisms present after the period of maximum growth, probably by controlling the reaction of the medium.

4. "Peptones" high in proteose content are apparently necessary for toxin production for other reasons than their buffering action.

5. More abundant growth was obtained with Difco-Proteose Peptone, either with or without increased carbon dioxide tension, than with Difco-Bacto Peptone.

6. No appreciable amounts of toxin were found during the period of most active growth, which is the period of decrease in amino acid supply. On the other hand, it seems to be formed after the period of maximum growth, and during time of increase of amino acid content.

7. These tests should help to explain the irregular results obtained in the production of diphtheria toxin when cultures of diphtheria organisms are grown under ordinary conditions.

This is a preliminary report.

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[2] Apt, G., et Loiseau, G., *Ann. de l'Inst. Pasteur,* 1925, xxxix, 114.

[3] Bunker, J. W. M., *J. Bact.,* 1919, iv, 379.

[4] Glenny, A. T., and Allen, K., *J. Path. and Bact.,* 1921, xxiv, 61.

[5] Kligler, I. J., *J. Bact.,* 1917, ii, 351.

[6] Römer, P. H., and Sames, Th., *Z. f. Immunitätsf.,* (Orig.) iii, 344.

[7] Wolf, C. G. L., *Biochem. J.,* 1922, xvi, 541.

3553

Testicular Lesions in Rabbits Following Intraspinal Syphilitic Infection.

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In the course of an investigation concerning the therapeutic efficiency of various chemical compounds in neurosyphilis, it became necessary for us to determine their trypanocidal and spirocheticidal effects in the cerebrospinal system. The central nervous system of animals was infected by the introduction of either trypanosomes or spirochetes into the intraspinal canal. After complete anesthesia of the rabbit is effected, a small incision is made in the parietal region of the skull and the animal then trephined with a small bore.* After trephination, a special capillary pipette is introduced into the opening, and the spirochetes or trypanosomes injected into the fourth ventricle by means of a syringe. After the introduction of the microorganisms the incision is sutured, and after complete recovery from the anesthesia, which usually requires two or three hours, the chemical compounds are introduced intravenously into the animals. Our plan was to destroy the trypanosomes or spirochetes after they were introduced into the cerebrospinal system, by the action of small amounts of chemical compounds which were able to penetrate the choroid plexuses and reach the central nervous system. In this manner we were able to sterilize the spinal fluid by the use of various compounds.

The problem consisted in the selection of the most penetrative and at the same time the most effective spirocheticidal substance. It was necessary to develop a technic for the production of a definite infection of the cerebrospinal system, particularly the spinal fluid. In this article we will describe our results with spirochetic infections, while our experiences with trypanosome infections will be reported elsewhere.

Various investigators attempted to infect the central nervous system of rabbits with syphilis. Thus, Uhlenhut[1] tried to inoculate the brains of healthy animals with syphilis, but obtained no positive results. The attempts of Noguchi and Bartarelli[2] failed too. Plaut[3] inoculated rabbits intratesticularly with spirochetes obtained from the brain of a paretic, and observed certain changes in the cerebrospinal fluids of the animals which, in his opinion, furnished definite evidence of a syphilitic infection. Brown and Pearce[4] inoculated rabbits with spirochetes through the testicles, and were subsequently able to demonstrate the penetration of the spirochetes into the spinal canal by injecting some of the spinal fluid into the testicles of healthy animals and producing typical syphilitic lesions.

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* Description of pipette and details of method, see *J. Chemotherapy*, 1927, April.

In our investigation it was necessary to secure definite evidence of syphilitic infection of the cerebrospinal system after the introduction of spirochetes into the fourth ventricle of rabbits. Incidentally, interesting observations were made, which clearly indicate that such an infection takes place in the majority of cases. Of particular importance is our observation that in the greater number of animals the lesions appear in the testicles. The spirochetes, after their introduction into the intraspinal canal, apparently soon disappear from the spinal fluid, enter the body and are probably carried by the lymphatic system or the blood stream into various parts of the animal body, finally producing skin lesions.

In our inoculations we always used numerous spirochetes which were motile, as confirmed by preliminary dark field examinations. In some cases in which the spinal fluid was examined three to five days after the inoculation, no spirochetes could be found by dark field examinations. Experiences with trypanosomes and spirochetes convinced us that the mere examination of the blood stream or spinal fluid does not always reveal the presence of trypanosomes or spirochetes. More exact methods are necessary in such cases.

The next procedure, therefore, is to inoculate healthy animals with the spinal fluid supposedly containing spirochetes and observe the development of testicular lesions. This was done by us in various instances with negative results thus far. The results obtained with rabbit No. 1155 are particularly interesting. It was inoculated intraspinally on January 25th, 1927, killed on January 28th, and some of the spinal fluid injected into the testicle of rabbit No. 1163. This animal was observed until May 2, 1927 (a period of 89 days) but no testicular lesions appeared. This rabbit is still under observation.

Finally, we decided to study the systemic manifestations of syphilis in rabbits infected intraspinally with spirochetes. This resulted in the striking observation that the majority of animals develop syphilitic manifestations when infected by the intraspinal route only. Of even greater interest is the fact that practically all of the rabbits which exhibited systemic infections developed testicular lesions.

Thus, rabbit No. 781 which received spirochetes intraspinally on 10-8-25 exhibited a medium node in the left testicle 53 days after the inoculation. The dark field examination of the testicular tissue showed a large number of very active spirochetes. Rabbit No. 1069, inoculated intraspinally showed a small node in the right testicle 120 days after inoculation. The left testicle exhibited a very hard medium sized node. This was observed 166 days after the intraspinal infection. The right testicle on examination in the dark field showed numerous very active spirochetes. It is interesting to note that in these animals the lesions disappeared from both testicles 187 days after intraspinal inoculation. Rabbit No. 1070, infected by the intraspinal route, showed a small scrotal chancre on the right side 47 days later, 11-15-26. The scrotum then became normal and remained so for nine weeks. On 1-24-27, 117 days after intraspinal inoculation, a small node appeared. On the scrotum of the left testicle a small chancre was evident. Examination of the scrotal tissue in this case again showed numerous very motile spirochetes. All the lesions disappeared on 3-28-27, 80 days after intraspinal infection. Rabbit No. 1068 was inoculated on 9-29-26. A small node was observed in the right testicle on 3-14-27, 166 days after intraspinal inoculation. On 4-4-27, 187 days later, a large superficial chancre appeared on the scrotum of the right testicle. The left testicle showed a small node. Examination of the testicular tissue showed the presence of very numerous and active spirochetes. In this animal, 187 days after intraspinal inoculation, we also observed an ulcer about the size of a ten cent piece in the middle of the ventral part of the tail, and a small, very hard, pea-sized node on the right leg. Both contained numerous motile spirochetes.

From the above it is safe to conclude that the introduction of spirochetes into the cerebrospinal system through the fourth ventricle produces a syphilitic infection in a majority of cases in the rabbit. The presence of spirochetes cannot be proved by a mere dark field examination of the spinal fluid. It cannot always be confirmed by inoculation of the supposedly infected spinal fluid into the testicles of healthy rabbits, a procedure usually employed for such investigations. The best method is to observe the animals after the intraspinal inoculation of spirochetes over a long period of time. In our opinion this period should last from six to eight months. The animals should be examined very carefully because the lesions may be small and therefore overlooked. All lesions and even the tiniest node should be viewed under the dark field for spirochetes. The findings are usually positive. It is significant to note that in the majority of cases the intraspinal inoculation of rabbits results in the development of testicular lesions. The investigations of others, especially Brown and Pearce,[4] indicate that the spirochetes migrate from the site of the testicular inoculation into the intraspinal canal and generally to the cerebrospinal system. Our experiments, however, definitely establish the fact that they travel from the cerebrospinal system to the superficial tissues, particularly the testicles.

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[1] Uhlenhut, P., *Med. Klinik,* 1922, xviii, 1210, 1246.

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[3] Plaut, F., and Mulzer, P., *Muench. med. Wchnschr.,* 1922, lxix, 1779.

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3554

The Antirachitic Activity of Monochromatic and Regional Ultraviolet Radiations.

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In a communication published some years ago it was shown that ultra-violet rays longer than about 324$\mu\mu$ exert no antirachitic potency, and that waves 302$\mu\mu$ are of great value in this respect.[1] Somewhat later we suggested, as the results of experiments with selective filters, "that the intense line of the mercury vapor spectrum 302$\mu\mu$ in length conferred definite protection and that the 313$\mu\mu$ waves exerted probably a feeble action in this respect."[2] The question as to whether antirachitic potency ceases at the 313$\mu\mu$ or the 302$\mu\mu$ level is of both theoretical and clinical importance, as the shorter rays are present to but a small extent and in low intensity in the solar radiations which reach the surface of the earth. This is especially the case where the rays are intercepted by moisture or smoke. It, therefore, seemed worth while to make a more minute dissection of the spectrum in this region. This has been accomplished by means of the isolation of these two lines of the mercury vapor spectrum and testing their power to activate cholesterol. The radiations were of equal intensity, and the same amounts (2.5 mg. per capita daily) of the irradiated cholesterol were fed to rats which had been rendered rachitic. Without going into detail at this time, it may be stated that a series of experiments of this nature showed that the 302$\mu\mu$ line possessed marked antirachitic potency, whereas the 313$\mu\mu$ line exerted very slight specific power. This result emphasizes more strongly than ever the remarkable specificity of wave lengths of light in relation to rickets.

It has never been accurately determined whether radiations less than 290$\mu\mu$, the shortest emitted by the sun, have curative power in rickets. About two years ago the shorter ultra-violet waves were segregated from the longer radiations by means of a filter containing chlorine and bromine, which, however, allowed the passage of about 0.1 per cent of radiations between 290$\mu\mu$ and the visible blue.[2] Recently we were able to isolate two bands of radiations given off by the mercury-vapor lamp—those less than 290$\mu\mu$ in length and those between 290$\mu\mu$ and 313$\mu\mu$. Their antirachitic activity was tested directly by irradiating rachitic rats. It was found that radiations shorter than 290$\mu\mu$, in other words shorter than those produced by the sun, exert a more intense antirachitic effect than the most potent region of the solar spectrum. This result is in accordance with clinical experience.

This is a preliminary report.

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3555

The Protoplasmic pH of Living Cells.*

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The intra protoplasmic pH of Amoeba and of cells from different tissues of Necturus and the Frog was obtained by injecting with the micrurgical technique solutions of Clark and Lubs' indicator dyes. This method allows the use of dyes which cannot penetrate living cells from without, a situation which obtains for the majority of indicator dyes. The protoplasm tolerates only a limited amount of the injected fluid. If too much is injected the protoplasm

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* Expenses connected with this investigation were in part defrayed from a grant by the Ella Sachs Plotz Foundation.

undergoes visible disintegration, with the production of an acid which the dye immediately registers. As long as the cell remains alive the final tint in the protoplasm is always the same.

The Necturus and Frog were slightly anesthetized by immersion in etherized water. They were then removed and pieces of the various organs were carefully teased in blood serum. Determinations of pH were made on the cells of ciliated epithelium, gastric and intestinal mucosa, liver, pancreas, striated muscle, and unripe ova. The pH values obtained were the same for every cell: viz., 7.5 ± 0.1 for the nucleus, both normal and injured; 6.9 ± 0.1 for the normal cytoplasm; and 5.3 ± 0.2 for the injured and cytolyzing cytoplasm. These results are in close agreement with those obtained on the starfish egg.[1]

Variations of pH found by previous investigators may be accounted for by the fact that in most of the determinations crushed cellular tissue was used. The low pH values obtained were probably due to the acid produced by injury. On the other hand, the higher pH values can be explained by the presence of variable amounts of alkaline (pH 7.4 to 7.6) interstitial fluids and blood.

The pH of *Amoeba dubia* and *proteus* was also determined to be in the close neighborhood of 6.9 ± 0.1. Amebae, injected with phenol red, were maintained alive and well for over 48 hours without an appreciable change being noted in the color characteristic for the pH originally recorded. The pH value we obtained does not agree with the pH value of 7.6 obtained by the Needhams.[2] The Needhams based their results on the injection of neutral red, brom thymol blue, and phenol red. They do not mention the survival time after the injection of neutral red. They do state, however, that brom thymol blue killed the amebae within 2 to 3 minutes and phenol red, within 5 to 6 minutes.

In conclusion, it may be stated that the living protoplasm of widely differing types of cells, *i. e.*, echinoderm ova,[1] amebae, tissue and germinal cells of Necturus and the Frog, under normal conditions apparently have a remarkably constant pH value, which is slightly on the acid side, while the nuclear pH is distinctly on the alkaline side of neutrality. Injury produces an increased acid reaction in the cytoplasm but does not affect the pH of the nucleus.

This is a preliminary report.

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[2] Needham, J., and Needham, D. M., *Proc. Roy. Soc.*, London, Ser. B, 1925, xcviii, 259.

3556

Effect of Changes of Blood pH on Reaction of Heart to Cocaine.

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Changes in the concentration of hydrogen ions of the blood previously reported[1] produced well-marked effects in the reaction of the heart to caffeine. Other drugs under these conditions have since been studied, among them cocaine. Observations were made on the heart *in situ,* after removal of the anterior portion of the chest wall. The experiments were done on cats anesthetized with urethane, under artificial respiration. The contractions were recorded on smoked paper by means of levers attached with fine threads to the right auricle and right ventricle.[1]

The results obtained were in several respects much more striking than in the experiments with caffeine or the other drugs. The resistance of the heart to cocaine was greatly increased by the intravenous injection of alkali, and very much diminished by acid. Small doses of the alkaloid administered after the intravenous injection of weak acid caused pronounced depression of the heart. While alkali did not necessarily nor always prevent the action of cocaine, its injurious effect was greatly decreased and often entirely suppressed.

Experiment No. 1. Sodium carbonate in a concentration of 2.5 per cent was injected intravenously in divided doses, into a cat weighing 2.5 kilos, until a total of 0.9 gm. was given. The subsequent injection of 1 cc. of 1 per cent cocaine hydrochloride, or 4 mg. per kilo, failed to produce any noticeable change in the action of the heart. The same amount of the alkaloid, administered 33 minutes after 0.75 gm. sodium acid phosphate, given in a dilution of 2.5 per cent, decreased the frequency of the heart about 60 per cent, and greatly weakened the force of the contractions.

Experiment No. 2. A dose of 3 mg. cocaine hydrochloride, injected one minute after administration of 3 cc. of 1 per cent HCl acid, reduced the strength of the contractions 60 to 70 per cent and considerably decreased their frequency. The same amount of cocaine, given after the administration of 1 gm. Na_2CO_3, in a 2.5 per cent dilution, failed to produce the effect which was previously observed after acid. Four more doses of cocaine, in the same amount as before, produced no noticeable change in heart action.

The comparative toxicity of cocaine when the C_H of the blood is normal, and when the pH is increased by alkali, was shown in several instances, the following being a typical case.

The first dose of cocaine hydrochloride, 4 mg. per kilo, decreased the strength of the contractions in both auricles and ventricles, the effect being greater in the former than in the latter. Each pair of contractions of the auricles was also unequal. While the frequency was considerably reduced in both, the slowing was much greater in the ventricles than in the auricles. The rhythm was 5:2. A complete recovery of the ventricles occurred within two minutes but only partial improvement was observed at the end of this time in the auricles. Blood pressure in this, as in other experiments under similar conditions, was greatly decreased and followed the auricles. The same amount of cocaine, after the injection of 20 cc. 2.5 per cent Na_2CO_3 or 0.5 gm. per kilo, caused only a slight decrease of

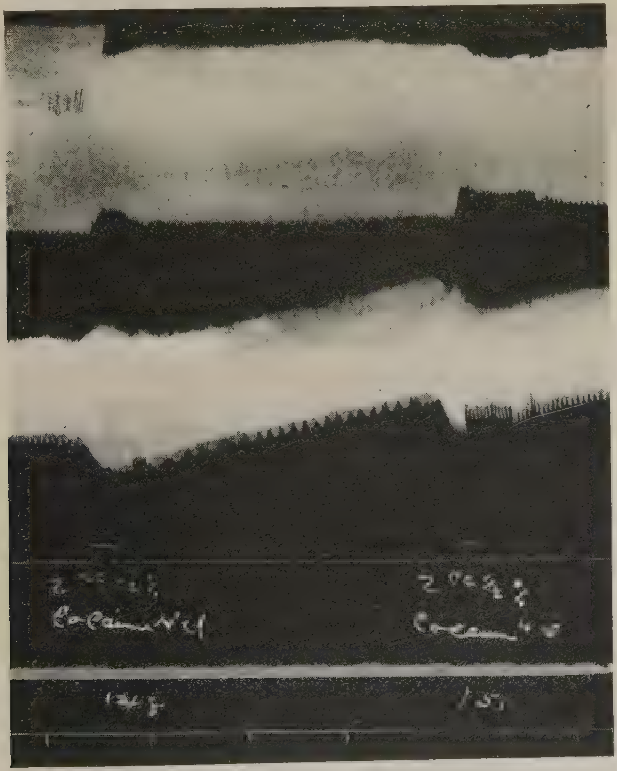

Fig. 1.

Vagi cut. First and second injection cocaine HCl. Dose 5 mg. per kilo. Thirty cc. 2½ per cent Na_2CO_3 administered before cocaine. Upper tracing auricle, lower ventricle.

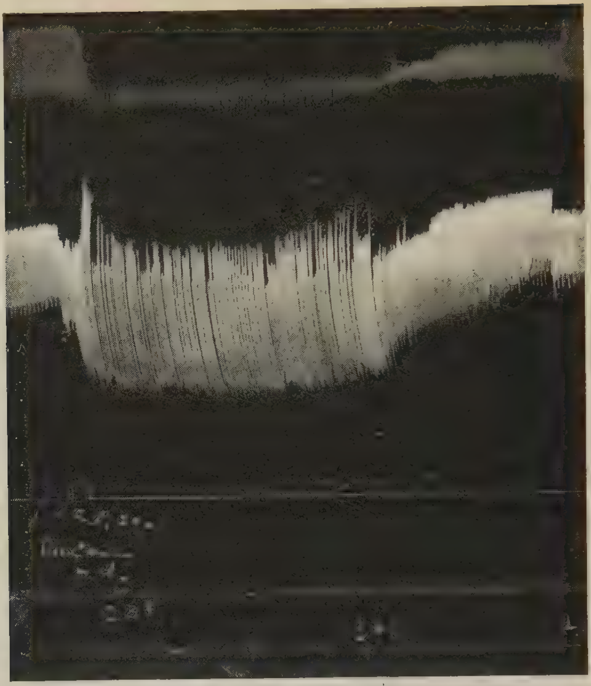

FIG. 2.

Vagi cut. Third injection of cocaine HCl. Dose 1 mg. per kilo. Total cocaine previously injected 3 mg. per kilo in 2 injections. Twenty-four cc. 1 per cent HCl administered before cocaine. Upper tracing auricle, lower ventricle.

the force of the auricular beats, while that of the ventricles was somewhat increased. The rate was not affected in either case. The fall of blood pressure amounted to a few mg. only. In this connection attention may be called to another experiment, in which the same amount of cocaine per kilo, administered after 8 cc. sodium acid phosphate, stopped the heart in about 50 seconds. The effect of changes in the C_H of the blood on the action of cocaine was demonstrated even more satisfactorily when larger amounts of acid and alkali were given at frequent intervals. After increased amounts of acid, the minimum toxic dose of cocaine was reduced to about half. One mg. per kilo was sufficient to cause depression of the heart, a single dose of 4.7 mg. per kilo caused paralysis. When the amount of acid was increased, cardiac paralysis was produced by a dose of 3.5 mg. cocaine per kilo.

The protection against cocaine, conferred by alkali received further corroboration in two experiments in which the pH of the blood was maintained at a high level by the frequent injections of Na_2CO_3.

The surely toxic or even fatal dose of cocaine could thus be given repeatedly without causing any significant effects on the heart. In one experiment doses of 5 mg. per kilo were injected until 85 mg. per kilo were administered. The effect of the drug became pronounced only after the fourth injection. Furthermore, when the last dose was given, the auricles, though depressed, were still contracting, while the ventricular beats were quite vigorous. The resistance to cocaine was still greater in another cat. The total amount of the drug tolerated was 105 mg. per kilo, which was administered in divided doses in one hour and 41 minutes. Heart action was fairly good even after the last dose, while distinct evidence of toxicity was first obtained only after eight doses were given, or a total of 40 mg. per kilo.

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[1] Salant, William, and Nadler, J. Ernest, *Am. J. Physiol.*, 1926, lxxviii 308.

3557

Feeding Experiments With Plants at Different Stages of Development. III: Synthesis of Vitamin in Plants.

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In a previous publication[1] we have pointed out that in order to determine whether there is a synthesis of vitamin during the germination and greening of corn, it is not enough to compare *equal weights of the dried material* (ungerminated, germinated and green), as other workers have done before us, but to compare *an equal number of seeds.* This we have now done, in a continuation of our experiments. Fifteen rats were kept on a synthetic diet, deficient in vitamin A, until they became stationary in weight. To the diet of the first five (group A) was now added the equivalent of six seeds of ungerminated corn per rat per day; to the diet of the second group of five (group B) was added the equivalent of six seeds of germinated corn per rat per day; to the diet of the third five (group C) was added the equivalent of six seeds of green seedlings per rat per day. Within 81 days after the corn feeding had begun, all the rats in groups A and B had contracted xerophthalmia, and 8 had died while all the rats in group C were in excellent condition and were continuing to gain in weight.

The average weight of each seed was for the ungerminated, 0.17 g., for the germinated, 0.16 g., and for the green 0.11 g. Despite the fact that the weight of green material offered group C was less than the weights of germinated and ungerminated material offered groups A and B, respectively, group C thrived, whereas rats of groups A and B developed xerophthalmia and declined..

Since vitamin A is associated with xerophthalmia, these results show conclusively that there was a synthesis of vitamin A during the course of greening.

This is a preliminary report.

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3558

Changes in CO_2 Combining Capacity of Blood Following Exercise in Individuals with Organic Heart Disease.

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(Introduced by R. H. Halsey.)

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Changes in the carbon dioxide combining capacity of the blood in normal individuals following vigorous exercise have been reported by a number of investigators. These changes consisted chiefly of a decrease in this constituent. Barr, Himwich, and Green[1] found that easy muscular exercise may be performed with little or no change, but that with heavier work the degree of change in carbon dioxide combining capacity increases rapidly with each small increase in the amount of work.

The purpose of this investigation was to determine whether or not similar changes in the carbon dioxide combining capacity occur in individuals with organic heart disease after performing a definite amount of work. The majority of the cases studied were children between the ages of 8 and 16 years, with organic heart disease caused by the infection of rheumatic fever. We also made observations on two normal individuals, and one case of congenital heart disease. The exercise consisted of climbing thirty feet of stairs in forty seconds. In a number of milder cases, the effect of climbing sixty feet was studied. The patients were ambulatory, but varied in their ability to carry on physical activity. The cases were classified according to the classification adopted by the American Heart Association: Class I. Organic—able to carry on habitual physical activity. Class II. Organic—able to carry on: A. Slightly diminished physical activity; B. Greatly diminished physical activity. Class III. Organic—unequal to any physical activity. Class E. Possible—having doubtful murmurs, mainly accidental, possibly organic. Class F. Potential—having a predisposing history.

Blood was taken from a cubital vein without pressure after the patients had rested at least one half hour, and again approximately three minutes after the exercise was completed. The carbon dioxide combining capacity was determined immediately by means of Van Slyke's apparatus.

It will be noted from Table I that the two normal individuals and the three Class I cases did not show any decrease in the CO_2 combining capacity following the exercise, but a small increase. Of the seven cases which were classified as II A, three presented an increase, and four a decrease in this constituent. The decrease in subject 12 is quite marked. Of the six Class II B cases, three showed a slight increase, and the remainder a marked decrease in the CO_2 combining capacity. It is quite obvious that decreases in the CO_2 combining capacity occurred only in the Class II A and Class II B cases, and that the greatest decreases were noted in the

TABLE I.

Changes in CO_2 Combining Capacity of Venous Blood in Individuals with Organic Disease after Exercise (Stair-climbing—30 ft. in 40 sec.).

Subject	Class	CO_2 Combining Capacity			Diagnosis
		Before Vol.%	After Vol.%	Difference Vol.%	
1. L. D.		56.7	57.6	+0.9	No disease.
2. M. K.		61.7	62.1	+0.4	No disease.
3. S. B.	I	65.3	69.1	+3.8	Mitral insufficiency.
4. M. B.	I	62.4	62.4	0.0	Aortic & mitral insufficiency
5. M. D.	I	68.1	69.1	+1.0	Mitral stenosis & insufficiency
6. R. K.	IIA	61.4	74.8	+13.4	Aortic & mitral insufficiency
7. S. B.	IIA	60.5	62.4	+1.9	Aortic & mitral insufficiency
8. J. K.	IIA	60.5	66.2	+5.7	Aortic & mitral insufficiency
9. V. M.	IIA	66.2	65.3	—0.9	Mitral stenosis & insufficiency
10. B. C.	IIA	57.6	55.7	—1.9	Congenital dextrocardia
11. P. L.	IIA	72.9	68.1	—4.8	Mitral insufficiency
12. T. L.	IIA	70.0	62.4	—7.6	Aortic & mitral insufficiency
13. M. B.	IIB	60.5	63.3	+2.8	Mitral stenosis & insufficiency
14. F. R.	IIB	64.3	65.3	+1.0	Mitral stenosis & insufficiency
15. R. N.	IIB	61.4	64.3	+2.9	Aortic insufficiency, mitral stenosis
16. J. H.	IIB	65.3	55.7	—9.6	Aortic insufficiency
17. A. M.	IIB	71.0	60.5	—10.5	Mitral stenosis & insufficiency
18. A. N.	IIB	64.3	56.7	—7.6	Aortic insufficiency, mitral stenosis

Class II B cases, although all the cases performed the same amount of exercise. Increases were noted in cases from all three classes.

In Table II are presented the changes observed in CO_2 combining capacity after the children climbed sixty feet of stairs. These changes are compared with those occurring after climbing thirty feet. The cases studied were taken from those showing no decreases in this constituent after climbing thirty feet. It will be ob-

TABLE II.

Changes in CO_2 Combining Capacity of Venous Blood after Different Amounts of Exercise (stair-climbing).

Subject	Class	CO_2 Combining Capacity			Character of Exercise	Diagnosis
		Before Vol.%	After Vol.%	Difference Vol.%		
L. L.		68.1	67.2	—0.9	60 ft. in 120 sec.	No disease
J. B.	I	65.3	69.1	+3.8	30 ft. in 40 sec.	Mitral insufficiency
		59.5	63.3	+3.8	60 ft. in 125 sec.	
M. D.	I	68.1	69.1	+1.0	30 ft. in 40 sec.	Mitral stenosis and insufficiency
		64.3	61.4	—2.9	60 ft. in 125 sec.	
J. K.	IIA	60.5	66.2	+5.7	30 ft. in 40 sec.	Aortic and mitral insufficiency
		63.3	65.3	+2.0	60 ft. in 120 sec.	
V. M.	IIA	66.2	65.3	—0.9	30 ft. in 40 sec.	Mitral stenosis and insufficiency
		64.3	59.5	—4.8	60 ft. in 122 sec.	
M. B.	IIB	60.5	63.3	+2.8	30 ft. in 40 sec.	Mitral stenosis and insufficiency
		60.5	56.7	—3.8	60 ft. in 125 sec.	
F. R.	IIB	64.3	65.3	+1.0	30 ft. in 40 sec.	Mitral stenosis and insufficiency
		62.4	56.7	—5.7	60 ft. in 120 sec.	

served that the normal individual showed only a very slight decrease. One of the Class I cases presented no change, the other a slight decrease. One of the Class II A cases showed very little change, but the other presented a moderate decrease. Both Class II B cases showed a moderate decrease.

The results suggest that after performing an equal amount of work, the majority of the cases with severely damaged hearts will show a definite decrease in the CO_2 combining capacity, whereas the normal individual or the patient with a mild degree of heart damage will not present the same degree of change in this constituent. These observations suggest that the determination of the CO_2 combining capacity may be of value in the classification of patients with damaged hearts.

This is a preliminary report.

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[1] Barr, D. P., Himwich, H. E., and Green, R. P., *J. Biol. Chem.*, 1923, lv, 495.

3559

Studies on the Life History of Blepharisma undulans.

LORANDE LOSS WOODRUFF.

From the Osborn Zoological Laboratory, Yale University.

Studies on the life history of *Blepharisma undulans* have been in progress for over five and a half years, all the animals employed being the progeny of a single individual isolated November, 1921. The work was planned to determine, in particular, the viability of this infusorian in the absence of fertilization, and the effects of fertilization on the rate of reproduction, in an attempt to broaden the results from our earlier studies on *Paramecium aurelia*[1] and *Spathidium spatula*.[2] A preliminary statement of the characteristics of this race of *Blepharisma undulans* was presented in 1922.[4]

Whereas earlier workers on this organism found that conjugation occurred rather infrequently and invariably was followed by death, conjugation was readily induced in our pedigree cultures, and the exconjugants proved to be viable in nearly every case. Accordingly excellent material was afforded for the problem at hand.

Two conclusions may be emphasized from the data secured to date:

(1) The animals of this race of *Blepharisma undulans* may reproduce apparently indefinitely without recourse to conjugation; there being no "cycles" in the vitality of the organism, as evinced

by the division rate or otherwise, which terminate in death when fertilization is prevented.

(2) Animals isolated from a pedigree line and allowed to conjugate immediately show a markedly higher rate of reproduction than that of the parent line, and this accelerated division rate is maintained for at least thirty days and, usually, until the experiment is discontinued.

In brief, the conclusions derived from Paramecium[1] and Spathidium[2, 4] that these infusorians are capable of unlimited reproduction, under favorable environmental conditions, without recourse to fertilization may now be extended to Blepharisma; and, furthermore, the conclusion that fertilization accelerates the division rate, as previously demonstrated in Uroleptus[3] and in Spahidium,[2] may now be extended to Blepharisma.

These studies have been aided by a grant from the Bache Fund of the National Academy of Sciences.

This is a preliminary report.

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[1] Woodruff, L. L., *Quart. Rev. of Biol.*, 1926, i, 436.

[2] Woodruff, L. L., and Spencer, PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 240, 303; *J. Exp. Zool.*, 1924, xxxix, 133.

[3] Calkins, G. N., *J. Exp. Zool.*, 1919, xxix, 121.

[4] Woodruff, L. L., and Spencer, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 339.

3560

Inheritance of Atypical Form in Paramecium aurelia.

IMOGENE MOORE. (Introduced by L. L. Woodruff.)

From the Osborn Zoological Laboratory, Yale University.

In all of the lines of a pedigree culture of *Paramecium aurelia*, which had been derived from an animal isolated from the Woodruff culture, in approximately the 12,000th generation, and bred on beef extract in the usual way, there appeared animals showing a "lump" on the peristomal side of the posterior end.[1] During the next week similarly atypical animals appeared in a hay infusion mass culture, which had been seeded with animals from the above lines three months prior to the appearance of the "mutation", and also in the lines of a second pedigree culture derived from an animal isolated from this mass culture.

These atypical animals appeared healthy and active and when isolated each gave rise to two daughter cells showing the lump. As a rule the lump was visible in both daughter cells before division was complete, although in a few cases, it was not apparent until several hours afterward. Furthermore the lumps showed considerable variation in size. With such variations, however, the character persisted in all of the lines, none of them ever again showing a perfectly typical animal.

In the course of time the vitality of the animals became slightly lowered, and at the end of 25 days some of the lines died, having attained from 16 to 24 generations. The remaining lines are still in pedigree, having retained the lump for 30 generations, or 2 months. In the latter lines at least one definite period of endomixis has occurred without any apparent effect on the new character.

In brief, a new character has arisen, in a manner unknown, almost simultaneously in a number of different though related lines and has been inherited, so far, through 30 generations, involving one endomictic period.

This is a preliminary report.

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[1] Woodruff, L. L., *Quart. Rev. of Biol.*, 1926, i, 436; Dawson, J. A., *J. Exp. Zool.*, 1926, xliv, 133.

3561

Further Observations on the Precipitable Substances of B. typhosus and B. paratyphosus B.

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In preceding notes[1] two different precipitable substances derived from *B. typhosus* were mentioned which, according to the high N content and digestibility by trypsin seemed to be of protein nature and different from the trypsin resistant antigen of Douglas and Fleming. When a solution prepared according to the directions of these authors was precipitated with alcohol, first in alkaline, then in acid solution, a product was obtained almost biuret free and yielding much reducing sugar on hydrolysis. A substance with similar properties was prepared as follows: Typhoid bacilli were extracted with N/2 NaOH for 1½ hours at 37°, the solution adjusted to slight alkalinity, centrifuged and the supernatant fluid precipitated with alcohol. The precipitation with alcohol was repeated once in alkaline and once in acid solution removing each time some insoluble material. A further precipitation was made with barium hydroxide solution. The substance gave faint or negative protein reactions and yielded about 40 per cent reducing sugar (calculated as glucose) after heating for 5 hours with N/2 HCl. It reacted intensely with common immune sera for *B. typhosus* and for *B. enteridis* Gärtner, and with sera prepared with the antigen of Douglas and Fleming, slightly, if at all, with an immune serum for *B. paratyphosus B*. Negative or very feeble reactions were obtained with immune sera for the two preparations from *B. typhosus* mentioned above.

A typhoid immune serum absorbed with alcohol treated bacilli, still containing the so-called large flaking agglutinins (Weil and Felix) was without action on any of the above three preparations. It would seem, therefore, that they all may be different from the labile agglutinogen, supposed to be connected with the agglutination of the flagelli (Smith and Reagh,[2] Beyer and Reagh[3]).

The precipitable substance of *B. paratyphosus B* previously obtained by extraction with dilute alcohol was since prepared in another way. The bacilli washed with saline and 95 per cent alcohol and extracted with hot alcohol were heated for two hours with saline solution on the steam bath. After centrifugalization the saline solution was acidified and the precipitate removed. The supernatant fluid was precipitated in alkaline solution (1/10 N) with 1 to 2 volumes of alcohol. The material was dissolved and again precipitated with alcohol in alkaline and in acid solution. The yield amounted to 10 to 20 mg. per Blake bottle. In the present state the preparation was almost protein free, gave the values C 43.82 per cent, H 6.51 per cent, N 1.86 per cent, P 2.06 per cent. On hydrolysis for 4 hours it yielded 56.1 per cent reducing sugar, calculated as glucose.

This is a preliminary report.

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[1] Landsteiner, K., and Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 379, 602.

[2] Smith, Th., and Reagh, A. L., *J. Med. Res.*, 1903, x, 89.

[3] Beyer, H. G., and Reagh, A. L., *J. Med. Res.*, 1904, xii, 313.

3562

Rotation of Lactic Acid Produced by L. acidophilus.

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L. acidophilus has been found to produce dextro-rotary lactic acid from lactose when grown in pure culture in casein-digest broth. After an acidity equivalent to 0.6 cc. of tenth normal sodium hydroxide per cc. had developed, it was neutralized by calcium oxide, evaporated under reduced pressure (not heating over 60° C.) to a syrupy consistency, strongly acidulated with concentrated sulfuric acid, filtered and extracted with ether in a continuous extractor. The ether was evaporated under reduced pressure and the syrup neutralized with zinc carbonate to form zinc lactate. This was precipitated with alcohol, filtered, dissolved in water and subjected to fractional crystallization.

Using a 5 per cent solution, a polariscope examination of three different fermentations, independently determined by each of us, gave a specific rotation of —2.5° for the second crop of zinc lactate. Therefore the lactic acid, which bears the opposite sign from its zinc salt, is dextro-rotary.

The work is still in progress.

3563

Influence of Peptic Digestion on Protective Colloid Value of Gelatin.

A. G. OLSEN AND M. S. FINE.

From the Research Department, Postum Company, Battle Creek.

Occasional allusion is made to the rôle of "protective colloids" in physiological processes. Commonly these are evaluated according to their "gold number", on which basis gelatins are conceded to be particularly powerful protective colloids (gold number 0.005 to 0.01).[1]

In connection with a series of digestion experiments we have had an opportunity to observe the influence of peptic digestion on the protective power of gelatin, as measured by gold number. These included both *in vitro* peptic digestion experiments, and observations upon gastric samples removed periodically from human subjects following gelatin meals.

The beaker experiments indicated that digestion with pepsin has no influence on the protective colloid value of gelatin. That digestion actually occurred was determined by precipitation with trichloracetic acid and subsequent determination of non-precipitable nitrogen, as well as by observing the gradual loss of viscosity and power to form gels. The digestion of a 10 per cent gelatin solution furnishes a striking example. The blank upon cooling set to a firm gel, while the digested mixture remained completely fluid. The gold number of the unchanged gelatin was 0.007, while that of the digested gelatin was 0.006. Evidently the peptic digestion products of gelatin possess essentially the same protective value as does the original gelatin.

In the *in vivo* experiments, the protective value of the gastric contents suffered a progressive decrease, due to the dilution caused by secretion and evacuation. However, the rate of diminution of protective colloid value together with the *in vitro* observations above noted would suggest that gelatin reaches the duodenum with its gold number essentially unchanged.

This is a preliminary report

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[1] Taylor, W. W., "The Chemistry of Colloids," p. 129.

3564

Rôle of Viscosity in Milk Modification.

A. G. OLSEN AND M. S. FINE.

From the Research Department, Postum Company, Battle Creek.

A few years ago Brennemann[1] reported a notable study concerning the coagulation of cows milk in the stomach, in the course of which he had occasion to study the influence on curd formation of a number of different types of milk modifications. He states "that nearly all of the numerous devices that are used in infant feeding in adapting cow's milk to the infant are directed, consciously or unconsciously to modifying this curd so that it will be fine, soft, and flocculent as is that of breast milk." Brennemann[1] found "that starch concoctions very radically influence the coagulation of milk in the stomach, and that the soluble carbohydrates, dextrins, milk sugar, cane sugar, and maltose have no appreciable influence." Colloid enthusiasts are prone to explain the clotting of milk and the influence of different milk modifiers in inappropriate colloid terms. Alexander[2] first used colloid terminology in explaining milk clotting and since then many writers have ascribed to "colloidal protection" the influence of such modifiers as gelatin, tapioca, starch, barley gruel, gum arabic, etc. Palmer and Richardson[3] have indicated certain fallacies in Alexander's original claims, but do not offer any alternative theory to account for the commonly observed influence of such milk modifiers.

In a series of experiments, including both *in vitro* and *in vivo* observations, we have found no relation whatever between protective colloid value, as measured by gold number, and influence upon the nature of the milk curd. We have, however, found a direct correlation between the *viscosity* of the modified milk and the fineness of curd produced, and feel warranted in concluding that the term "protective colloid" is not strictly applicable in this connection. The important property, which is involved in producing fine, soft, flocculent curds, appears to be viscosity rather than protective colloid value. For this reason a modifier which best maintains its viscosity unimpaired at body temperature, and under the conditions of gastric digestion may be expected to be most efficacious for the purpose. Tapioca was used as an example of farinaceous material of relatively low protective colloid value (measured by gold number) but of high viscosity. It proved to be an efficient means of obtaining fine soft curds.

This is a preliminary report.

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[1] Brennemann, J., *Arch. of Ped.*, Vol. xxxiv, Feb., 1917.

[2] Alexander, J., *J. Am. Chem. Soc.*, 1910, xxxii, 680-687.

[3] Palmer, L. S., and Richardson, G. A., *Colloid Symposium Monograph*, 1925, iii, 112-134.

3565

Identity of Precipitin and Complement Fixing Substances in Syphilitic Sera. I.

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Whether or not different types of antibodies are identical is still one of the classical questions in immunology. In this country, Zinsser[1] is the outstanding champion of the "unitarian" hypothesis.

The identity of precipitins and complement fixing substances in syphilitic sera is being championed in Germany, especially by Jacobstahl.[2] He was the first to show that the precipitate produced by extract antigen and syphilitic serum possesses the property of fixing complement, while the supernatant fluid remaining after removal of the precipitate lacks this complement fixing property.

The question of the identity of complement fixing and precipitin substances in syphilitic serum has recently been indirectly revived through the efforts of several workers to evolve a combined precipitation—complement fixation method in syphilis. Thus, in the case of the Sachs-Georgi test, Kafka,[3] Keining[4] and others have attempted to add complement to the resulting precipitate of this test and carry out a complement fixation reaction. The results, however, have been variable, due undoubtedly to the technical nature of the Sachs-Georgi reaction and the prolonged incubation which it requires.

It was believed that the Kahn test[5] might be suitable for the study of the identity of precipitin and complement fixing substances in syphilitic sera. This test gives immediate precipitation reactions on mixing serum with antigen. By adding the hemolytic system to the serum antigen mixture of the completed test, it was believed that immediate complement fixation reactions might also be obtained. If the complement-fixing and precipitin substances are identical, there should be parallelism between the complement fixation and precipitation results. If not identical, little or no parallelism should exist.

Procedure of combined precipitation-complement fixation test: Kahn tests were performed with syphilitic and non-syphilitic sera in the regular manner. After reading the results, 0.15 cc. amounts of the antigen-serum-saline mixture of the third tube of the test, containing about 0.035 cc. serum, were pipetted into a tube and 0.1 cc. (2 units) of complement added. The mixture was placed in the water bath at 37° C. for 30 minutes after which 0.1 cc. (2 units) amboceptor and 0.1 cc. sheep-cell suspension were added, and the mixture again placed in the water bath for about 15 minutes. When the serum and antigen controls showed complete hemolysis, the complement fixation results were read. Complete fixation of complement as well as complete precipitation was read four plus. Weaker reactions were read three, two and one plus, respectively. The usual serum, antigen and anticomplementary controls were employed.

Eighteen hundred sera, of which over a third came from syphilitic patients, were tested in this manner. It was found that 93 per

TABLE I.
Comparison of Precipitation (Kahn) and Complement Fixation Results with 1864 Sera.

Precipitation reactions	No. of examinations	Complement fixation reactions.		
		Positive	Doubtful	Negative
Positive	647	596	31	20
Doubtful	110	22	64	24
Negative	1107	17	12	1078

Percentage of agreement between the two methods: Complete, 93.24; relative, 4.77; disagreement, 1.98.

Positive reaction = ++++, +++ or ++.

Doubtful reaction = + or ±.

Complete agreement = positive or negative with both methods.

Relative agreement = positive or negative with one method and doubtful with the other.

cent of the sera gave complete agreement, being either positive or negative in precipitation and complement fixation procedures, 5 per cent of the sera gave relative agreement (Table I) while 2 per cent gave complete disagreement.

An attempt was then made to carry out quantitative precipitation-complement fixation studies.[5] Thus, after completing a quantitative Kahn test on a given syphilitic serum, 0.15 cc. amounts of the antigen-serum-saline mixtures from each tube were pipetted into a series of other tubes, complement added, incubated for 30 minutes and the complement fixation test completed as in the previous experiments. Of 80 sera tested in this manner, it was found that in practically all cases the highly potent sera showed an equally large number of precipitin and complement fixing substances, while the weakly potent sera showed a small number of such substances. The variations occurring were believed to be due to difficulties inherent in the hemolytic system. These studies are still being continued.

This study throws light on the probable identity of complement fixing and precipitin substances. It also appears that the complement fixation results obtained by adding the hemolytic system to completed Kahn tests would compare favorably with many of the Kahn-Wassermann studies reported in the literature. The combined procedure saves much time and material as compared with other complement fixation tests and might prove a popular method in laboratories where the Wassermann is used in conjunction with the Kahn test. Such a procedure should also be desirable when presenting to students the subject of precipitation and complement fixation with syphilitic sera.

Summary: We studied the identity of precipitin and complement fixing substances in syphilitic sera by adding the hemolytic system to completed Kahn tests to determine whether sera giving positive precipitation reactions would give positive complement fixation reactions. Of 1800 sera tested, it was found that 98 per cent gave either identical or approximate results with the precipitation and complement fixation methods. The remaining 2 per cent showed disagreement. This is believed to be due to technical factors rather than to lack of indentity of the "antibodies." The work is being continued with a view of reducing this variation.

It is believed that the combination of the hemolytic system with the Kahn test might offer a desirable complement fixation procedure in place of the Wassermann test in laboratories where it is employed parallel with the Kahn test.

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[1] Zinsser, H., "Infection and Resistance," 3rd Edition, McMillan, New York, 1923, p. 317.

[2] Jacobstahl, E., Chapter in Carl Bruck: Serodiagnose der Syphilis, Julius Springer, Berlin, 1924, p. 305.

[3] Kafka, V., *Dtsch. med. Wochenschr.*, 1921, xlvii, 269.

[4] Keining, E., *Dtsch. med. Wochenschr.*, 1921, xlvii, 157.

[5] Kahn, R. L., "Serum Diagnosis of Syphilis by Precipitation," Williams and Wilkins, Baltimore, 1925, p. 148.

3566

Relation of Internal Secretion of Ovaries to Cholesterol Metabolism.

LOUIS BERMAN.

From the Biochemical Laboratory of Columbia University at the College of Physicians and Surgeons.

Pig ovary follicle fluid injected into normal female cats caused a decrease in the blood cholesterol. This decrease did not occur in male cats. Ovariectomized cats showed an increase in the blood cholesterol. This increase can be reduced by the injection of pig ovary follicle fluid and also by ovarian extracts shown to be active by the vaginal smear method. This work shows that the ovarian hormone regulates the cholesterol content of the blood. Ovarian extract injected into male animals does not influence the cholesterol content of the blood. The results are to be made use of as a chemical method of standardizing the activity of the ovarian hormone in extracts.

This is a preliminary report.

Iowa Branch.

State University of Iowa, April 28, 1927.

3567

Observations on Life-Span of Epinephrectomized Cats.

E. L. COREY. (Introduced by W. W. Swingle.)

From the Osborn Zoological Laboratory, Yale University.

In a previous communication[1] it was reported that the oral administration of considerable quantities of fluids to bilaterally epinephrectomized cats prolongs life for some days beyond the usual life-span. Further observations are presented in this report.

I. A series of four adult male cats were bilaterally operated (interval of seven days between operations) and given 50 cc. distilled water three times a day and 50 cc. of milk once daily. The average survival period was 143.8 hours.

II. A second series of cats, consisting of four non-pregnant females and two males, were given intravenous injections of 10 cc. of 7 per cent gum acacia in 10 per cent glucose every forty-eight hours, or at times when marked symptoms of adrenal insufficiency developed. The average survival was 132.8 hours.

III. A third series of seven animals (five non-pregnant females and two males) were treated with 10 cc. of a 5 per cent solution of sodium bicarbonate every forty-eight hours, or at times when marked symptoms of adrenal insufficiency appeared. The average survival period was 132.5 hours.

IV. Series four consisted of three female cats (one in advanced pregnancy, the remainder non-pregnant). The animals were given 10 cc. of a 5 per cent solution of sodium bicarbonate added to a 7 per cent solution of gum acacia in 10 per cent glucose, every forty-eight hours. The average survival period was 172.3 hours.

V. Four double-operated cats were kept at an average temperature of 7.4° C. following removal of the second adrenal. Since double operated animals appear to be very sensitive to cold, an experiment was undertaken in which such animals were kept at cool temperature. However, no effect was noted since the average survival period for the group was 126.3 hours.

VI. Four adult animals, consisting of one non-pregnant female and three males, were kept at an average temperature of 30° C. throughout the experiment. The average survival period was 194.1 hours. No food or fluids of any kind were forced upon the cats of experiments V and VI.

The conclusion seems evident that the average survival period for bilaterally epinephrectomized cats, when they are not subjected to repeated bleeding for blood tests, is in the neighborhood of 4 to 6 days. When double operated cats are bled repeatedly, especially after adrenal insufficiency symptoms develop, the period of survival is very materially shortened. Swingle[2] has recently reported that the blood findings (pH, CO_2 capacity, phosphorus, etc.) of cats surviving bilateral adrenal removal (4 to 6 days) are identical with those showing symptoms at sixty hours.

This is a preliminary report.

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[1] Corey, E. L., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 206.

[2] Swingle, W. W., *Am. Naturalist*, 1927, lxi, 132.

3568

Effects of Pituitrin Administration on Distribution of Injected Fluid.

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Although it is well known that the administration of extracts of the posterior lobe of the pituitary gland may affect blood volume and urine output, there is little agreement as to the mechanism involved. This is due in part to the varying dosages, modes of administration, time of observation, and condition of the experimental animal employed by the various investigators.

The purpose of this investigation was to study the effect of pituitrin administration on the distribution of injected fluid between the blood, urine and tissues. Normal dogs deprived of food and water for 20 hours were used as experimental animals. Ringer's solution was injected intravenously by means of a Woodyatt pump, for a period of two hours, at the rate of either 15 or 25 cc. per kilo body weight per hour. At times, from .05 to .08 cc. of commercial pituitrin (Parke, Davis & Co. or Eli Lilly & Co.) per kilo body weight per hour was added to this solution. Ether was administered by the open cone method in several experiments, in order to study the combined effects of pituitrin and anesthesia. The periods of observation included the two hours of injection and a post-injection period of three hours. Urine was collected by continuous catheterization. Blood was drawn from the jugular vein before, at the end of the injection, and at the close of the post-injection periods. Blood volume increases were calculated from the Hb. and serum protein changes. For comparative purposes 7 per cent of the body weight was considered the average preliminary blood volume. The small volume of urine that the animals would have passed without fluid injection has been neglected in these calculations. The percentage of injected fluid entering the tissues was calculated from the difference between the amount injected and that found to be in the blood and urine.

TABLE I.
Distribution of Fluid Injected.

Nature of Injection	Percentage excreted during injection period	Percentage found in blood stream*	Percentage calculated to enter the tissues*	Percentage excreted in three hour post-injection period
Avg. Ringer's	22.3	25.7	52.0	29.9
Avg. Ringer's and pituitrin	23.1	56.4	20.5	55.7
Avg. Ringer's and pituitrin and ether anesthesia	26.9	9.2	63.9	30.9
Experiments on 2 Nephrectomized Dogs.				
Avg. Ringer's	0	25.8	74.2	0
Avg. Ringer's and pituitrin	0	61.6	38.4	0

*At end of injection period.

Table I summarizes the average values obtained in 24 experiments on 10 animals. The effect of pituitrin administration has been to decrease greatly the amount of fluid entering the tissues. Ether anesthesia is antagonistic to this action and permits even greater quantities of fluid to be distributed to the tissues than in the control experiments with Ringer's solution. The values obtained in the different experiments were consistent, in that animals receiving pituitrin exhibited a greater increase in blood volume than the controls. This confirms the findings of Underhill and Pack[1] and other investigators. Approximately the same degree of difference was noted in experiments on nephrectomized animals. When the injections were made under ether anesthesia only a slight increase in blood volume was noted. Pituitrin administration was found to be without effect on the urine output during the injection period, but was followed by a greater output in the three hour after-period. We believe the latter effect to be due, not to the action of pituitrin *per se,* but rather to the increased fluid content of the blood present at the time pituitrin administration ceases.

This is a preliminary report.

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[1] Underhill, F. P., and Pack, G. T., *Am. J. Physiol.*, 1923, lxvi, 520.

3569

A Comparative Study of the Extent of the Knee-jerk and the Achilles-jerk.

W. W. TUTTLE. (Introduced by J. T. McClintock.)

From the Department of Physiology, State University of Iowa.

In this investigation the extent of the knee-jerk is compared with the extent of the Achilles-jerk. Apparatus has been devised which is capable of automatically delivering stimuli of uniform intensity, at a constant rate, to both the *ligamentum patellae* and the tendon Achilles.

In case of the knee-jerk, the subjects were seated in an adjustible chair so that they could be placed in proper proximity to the stimulating hammer. The leg was attached to a movable stylus, suspended from a rubber band, which records the lateral movement on a revolving drum. In all cases a constant leverage was maintained by keeping the point of attachment to the leg at a uniform distance of 30 cm. from the inferior margin of the *ligamentum patellae.* The details of the technique may be found elsewhere.[1]

For the Achilles-jerk experiments, the subjects were placed in a prone position on a well padded table, equipped so that the leg in question could not move. The apparatus is so devised that a stimulating hammer falls upon the tendon Achilles ten times per minute with a force of 296 grams. The extent of the movement of the toe is recorded by connecting it to a moveable stylus, as previously described. A constant leverage was maintained by keeping the point of attachment of the toe 26 cm. from the *malleolus medialis.*

The extent of the jerks was obtained by measuring the distance in millimeters through which the stylus moved when they were elicited. This distance is referred to as the "extent" or "height" of the jerk.

In this investigation 115 normal subjects were used. The data obtained from 95 subjects are shown in condensed form in Table I.

In the case of 20 subjects either one or both jerks was absent, and thus these data are treated separately.

For the purpose of comparison the data were divided into arbitrary groups, the limits of which are shown in column 2, Table I.

TABLE I.

A comparison of the distribution of subjects based on the extent of their knee-jerk with that of those based on the extent of their Achilles-jerk.

Group	Range mm.	No. K-J subjects in each group	Distribution of A-J subjects by groups							
			1	2	3	4	5	6	7	8
1	0-10	14	7	3	1	3	0	0	0	0
2	11-20	20	3	6	8	1	0	1	0	1
3	21-30	20	2	5	3	6	1	3	0	0
4	31-40	23	2	3	7	8	0	2	1	0
5	41-50	5	0	2	0	1	2	0	0	0
6	51-60	9	0	1	1	3	0	3	1	0
7	61-70	3	0	1	0	0	1	0	1	0
8	71-80	1	0	0	0	0	1	0	0	0

Seventeen of the subjects exhibited no Achilles-jerk, eleven of them falling in group 1, five in group 2, and one in group 3. In three cases both jerks were absent. The writer wishes to infer only that in the case of absent jerks subjects did not respond to the strength of the stimuli used, and not that it was impossible to elicit the jerks.

The data show a rather close correlation between the extent of the knee-jerk and the Achilles-jerk, especially in the lower groups where 81 per cent of the cases fall.

It is evident that where the knee-jerk is unusually high the Achilles-jerk falls into a lower group, which indicates a lack of proportional increase in extent.

Where the Achilles-jerk is absent, the majority of the cases fall into group 1, while the rest are found in groups 2 and 3. This seems to further indicate a close correspondence between the extent of the two jerks.

This is a preliminary report.

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[1] Tuttle, W. W., *Am. J. Physiol.*, 1924, lxxv, 338.

3570

Experimental Study of Ovarian Regeneration in Mice.

H. O. HATERIUS. (Introduced by W. W. Swingle.)

From the Zoological Laboratory, State University of Iowa.

Whether or not mammalian ovaries are capable of regeneration following complete ovariectomy seems still to be somewhat of a question. Davenport,[1] working on mice, found that, on the average, regeneration occurred in 64 per cent of his animals following removal of the ovaries. The experimental period ranged in time from 8 to 45 weeks, and he based his conclusions regarding ovarian regeneration upon the fact that post-mortem examination revealed distinct ovarian masses on the site of the original ovaries, and in many cases embryos were found *in utero*. No histological evidence was advanced, however, to show that all of the original ovary was removed in each case, and it seems essential that such evidence be established before it can be stated definitely whether regenerated ovarian tissue actually arises from the peritoneum, or from fragments of the original substance inadvertently left *in situ*.

Considering the importance of this subject in its bearing upon the problem of germ-cell origin, it has seemed desirable to repeat the experiment, basing any conclusions drawn upon histological study. Such evidence would show definitely two things: (1) whether or not all of the original ovary had been removed—a factor of prime importance—and (2) whether or not regenerated tissue actually is ovarian in nature.

Ninety-six operations were performed upon mice; 76 of the operated cases have been examined in connection with this report, having run over a period ranging from 7 to 16 weeks. Great care was exercised in removal of the ovaries, all of which were preserved for subsequent microscopic study to make certain that removal had been complete. The operative procedure was varied: in some cases the capsule was split and the ovary cut off at the hilus; in a majority of the operations, however, the capsule and hilus were included in removal; and in others a short section of the stalk was included as well. No cases of infection occurred, and subsequent examination showed little or no adhesions in the greater percentage of cases.

The results of examination are tabulated below, and show that in the great majority of cases (70 out of 76) no evidence of ovarian substance could be detected. Three cases showed a formation of bladder-like cysts on the site of the original ovary, but sectioning of the entire region showed no germ-cells or ovarian tissue; in another case a suspicious looking mass in the same position also proved negative. Six were found with regenerated masses which contained ovarian tissue; in checking up on the initial operation it was found that in two of these cases the original notes showed that complete removal of the ovary was doubtful. The remaining four cases of regeneration occurred, according to notes taken at time of the original operation, in animals from which the ovary was apparently entirely removed. However, in view of such a small percentage of positive cases (4 out of 76 operations) it seems highly probable that these cases can be attributed to incomplete ovarian extirpation. It is possible that microscopic bits of ovarian tissue lying in the hilus region may have hypertrophied to this extent.

TABLE I.
Summary of cases examined.

Interval between operation and examination	Number examined	No regeneration	Regeneration
7 weeks	2	2	
8 weeks	11	11	
9 weeks	38	35	3
10 weeks	16	14	2
11 weeks	1	1	
15 weeks	2	2	
16 weeks	6	5	1
	76	70	6

In addition to the ninety-six complete ovariectomies, five partial operations were performed, with the object in view of ascertaining to what extent regeneration occurs as a result of incomplete removal.

In each case a very small bit of ovarian tissue was left *in situ,* together with a greater portion of the capsule. Although these cases were allowed to run for only five weeks, examination revealed a remarkable degree of regeneration; in all five the regenerated mass approximated in size the average normal ovary. In each instance the capsule was markedly thicker than usual, and was very prominently supplied with bloodvessels.

The above evidence leads to the conclusion that, at least in an 8 to 16 week period, no regeneration occurs, provided the ovaries are completely removed. It is the writer's opinion that in mice ovarian tissue regeneration results from incomplete removal, and that such tissue does not arise *de novo* from non-gonadic tissue surrounding the site of the original ovary.

None of the mice examined was killed, and they will be kept alive over a considerable period, in order that the time-element may not be neglected as a possible factor in regeneration.

This is a preliminary report.

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[1] Davenport, C. B., *J. Exp. Zool.,* 1925, xlii, 1.

3571

Acid Intoxication of Adrenal Insufficiency in Dogs.

F. F. YONKMAN. (Introduced by W. W. Swingle.)

From the Zoological Laboratory, State University of Iowa.

Recent work on the blood chemistry of dogs and cats suffering from adrenal insufficiency suggests the probability that the adrenal cortex secretes a hormone which in some manner controls the normal functioning of the kidney. Several investigators[1] report marked nitrogen retention following adrenal removal. Swingle[2] and Swingle and Eisenmann[3] demonstrated phosphorus retention, and the existence of an uncompensated non-volatile acidosis in severe adrenal insufficiency. The acidosis was considered to be due to retention of the acid end products of normal metabolism.

Owing to the difficulty of bleeding cats repeatedly, and the fact that considerable quantities of blood are necessary for analysis, the writer made a detailed investigation of the acid intoxication following adrenal removal in dogs.

Adult male and non-pregnant dogs were employed, an interval of 5 to 10 days elapsing between operations. Longer intervals were not found to prolong the survival period, the average survival period to date being 5½ to 6 days. Dogs living less than 4 days were entirely disregarded for this investigation. No greater difficulty was experienced with the right gland than with the left.

All blood for CO_2 capacity and pH was drawn under oil, directly from the heart. Normal blood was drawn at first from unilaterally operated animals since this blood presents no change from normal.[4] Later, however, normal blood was obtained after the second operation. Double-operated animals are always normal for several days following the second operation. They eat, play, and fight with one another just as do unoperated animals. The blood picture of such animals shows no deviations from the normal. Following the onset of the characteristic adrenal insufficiency symptoms, blood was taken at various intervals.

The CO_2 capacity was determined by Van Slyke's method,[5] and the pH by means of Myer's bicolorimeter,[6] using Muntwyler's[7] correction of 0.30 for dog plasma.

Acidosis. The blood findings show that with the onset of symptoms of adrenal insufficiency such as refusal of food, muscular weakness and general lassitude the initial fall in the CO_2 capacity occurs, whereas, the pH remains normal. With continued fall in CO_2 capacity the pH remains normal until the capacity has fallen to approximately 30 volumes per cent. At this level the first decline in pH is observed, and when the level of the CO_2 capacity falls below 30 volumes per cent, a rapid decline in pH ensues. The gradual decrease in CO_2 capacity is accompanied by a corresponding increase in depth, and in the later stages, in the number of respirations. There comes a time, however, when respiratory compensation is inadequate and a sharp decline in pH follows, this change occurring between 4 and 12 hours preceding death.

The shifting of compensated acidosis to the uncompensated condition has been commented upon by Swingle[8] and the similarity to the acidosis of chronic interstitial nephritis has been cited.

Blood Sugar. With the onset of symptoms the initial fall in CO_2 capacity is accompanied by a marked drop in blood sugar which falls to approximately 75 mg. per 100 cc. The sugar level may fluctuate materially once it has begun to fall and in 2 of the several cases came back to normal. However, it invariably drops in the terminal stages preceding coma, in some cases to as low as 55 to 60 mg. per 100 cc. It seems probable that the early symptoms of adrenal insufficiency such as anorexia and lassitude are due to the initial decline in alkali reserve and blood sugar.

TABLE I. *Blood findings following bilateral suprarenalectomy in dogs.*

Animal	Date	Time	CO_2 capacity	pH	Sugar mg./100 cc.	Phosphorus mg./100 cc.	Remarks
Dog 1.	Feb. 4-27						Right suprarenal removed.
	” 11	8:30 p. m.					Left suprarenal removed.
Female	” 13	10:00 a. m.	44.40	7.36	95	4.25	Normal. Ate well.
Terrier	” 15	10:00 a. m.	41.40	7.37	77		Venous.* Refused food. Vomited. Moody.
	” 15	2:00 p. m.	33.10	7.32			Respiration—24/min. Deeper.
	” 15	9:30 p. m.	28.60	7.27	83	6.75	Resp.—24/min. Deep with sighs.
	” 16	5:00 a. m.	24.40	7.19	62	13.00	Prostrate. Died at 8 a. m.
Dog 2.	Feb. 12-27						Right suprarenal removed.
	” 17	2:00 p. m.					Left suprarenal removed.
Female	’ 19	10:00 p. m.	46	7.43	109	5.3	Normal. Ate meat. Drank milk.
Collie	” 22	10:00 a. m.	38	7.42	67		Refused food. Moody and weak.
	” 22	10:00 p. m.			78		Resp.—20/min. Vomiting.
	” 22	12:00 p. m.	37	7.40			Weaker. Resp.—24/min. Deep.
	” 23	3:30 a. m.	31(29)*	7.17	64	10.00	Venous. Restless. “Flat.” Died at 4 a.m.
Dog 3.	Mar. 2-27						Right suprarenal removed.
	” 12	4:00 p. m.					Left suprarenal removed.
Female	” 14	9:30 p. m.	40.3	7.38	111.1	4.72	Normal. Eating and drinking.
Spitz	” 15	9:30 p. m.			100		Muscle twitchings.
	” 16	4:00 a. m.	37.4	7.32	80		No food. Hind legs weak.
	” 16	9:00 a. m.			93		Cerebellar symptoms. Swaying.
	” 16	2:15 p. m.	37	7.34	94		Ate meat. Muscle twitchings.
	” 17	10:00 a. m.	35	7.33	108		Ate. Yelling fits. Resp.—shallow.
	” 18	9:30 a. m.	27	7.27	76.8		No food. Legs very weak. Resp.—14/min. Deep.
	” 18	7:15 p. m.	22	7.10	66	11.12	Venous.* Resp. deep and full—26/min. Died at 8:15 p. m.
Dog 4.	Apr. 9-27						Right suprarenal removed.
	” 14	3:00 p. m.					Left suprarenal removed.
Male	” 16	10:00 a. m.	45	7.41	91	3.1	Normal dog.
Mongrel	” 18	2:30 p. m.	34	7.42	78	5	No food. Resp.—16/min. Deep. Lying around. Moody.
	” 18	8:00 p. m.			77	6.25	Vomiting. Resp.—still 16/min.
	” 19	10:30 a. m.	32		77		Deep respiration. Very moody.
	” 20	4:00 p. m.	28	7.16	62	7.5	Prostrate. Died at 5:30 p. m.

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* Venous blood is two vol. per cent higher than arterial.

Phosphorus. The inorganic phosphorus of the serum shows a gradual rise with the onset of symptoms but the rise is most rapid in the later stages. This increase in phosphoric acid is not sufficient, however, to account for the degree of acidosis. Other nonvolatile acids undoubtedly play an important rôle. The data obtained from an extensive series of dogs clearly indicate that the cause of death following adrenal removal is acid intoxication, which appears to be due, in part at least, to failure of the kidney to eliminate certain of the acid end products of normal metabolism.

Table I shows data on four typical cases and is an attempt to correlate the corresponding deviations of the CO_2 capacity, pH, blood sugar and phosphorus with the visible characteristic symptoms.

This is a preliminary report.

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[1] Swingle, W. W., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 208; Stewart, G. M., and Rogoff, J. M., *Am. J. Physiol.*, 1926, lxxviii, 3,711; Lucas, G. H. W., *Am. J. Physiol.*, 1926, lxxvii, 1, 114.

[2] Swingle, W. W., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 208.

[3] Swingle, W. W., and Eisenmann, A. J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 212.

[4] Swingle, W. W., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 208; Lucas, G. H. W., *Am. J. Physiol.*, 1926, lxxvii, 1, 114.

[5] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1917, xxx, 289.

[6] Myers, V. C., *J. Biol Chem.*, 1922, liv, 675.

[7] Myers, V. C., and Muntwyler, E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, this issue.

[8] Swingle, W. W., *Am. Naturalist*, 1927, lxi, 132.

3572

Colorimetric Estimation of Hydrogen Ion Concentration of Blood.

VICTOR C. MYERS AND EDWARD MUNTWYLER.

From the Biochemical Laboratory, State University of Iowa, Iowa City.

Recently Myers, Schmitz and Booher[1] described a simple microcolorimetric technique of estimating the hydrogen ion concentration of the blood plasma, based upon an adaptation of the colorimetric method of Cullen[2] to the Myers bicolorimeter.[3] The pH readings are obtained on diluted plasma at room temperature and must be corrected to the actual pH of the plasma at body temperature by a constant. This constant includes corrections for dilution, temperature and protein error. The use of Cullen's factor of 0.22 is made in the following formula:

$$\mathrm{pH}_{38}{}^{\circ} = \mathrm{pH}_{t}{}^{\circ} + 0.01\ (t^{\circ}—20^{\circ})\ —0.22.$$

This factor serves very well for normal human plasma. However, recently considerable doubt has arisen as to its applicability to abnormal samples.

Hastings and Sendroy[4] found that the correction used by Cullen for colorimetric determinations performed at room temperature is unnecessary when readings are made with the diluted plasma at body temperature. Austin, Stadie and Robinson[5] find that this is not consistently the case and report considerable variation between the true pH and the colorimetric value of the diluted plasma at 38°.

During the past year we have had the opportunity of studying the variation in the constants in miscellaneous hospital cases, many of whom were receiving alkali. Colorimetric values were obtained on separated plasma, both at room and body temperature, employing the method of Myers, Schmitz and Booher[1] with the introduction of certain modifications. Electrometric determinations on the undiluted separated plasma were made at 38°. Estimation of the CO_2 content was made in every case and wherever possible plasma chloride and total plasma protein figures were obtained.

In 81 samples of plasma the C correction (colorimetric pH at 20° —the electrometric pH at 38°) gives an average value of 0.226 pH. Assuming that 0.22 is correct, 50 per cent of the values are within ±0.02 pH of this value. In two cases there is a maximum deviation of 0.08 pH. The average C correction for dogs plasma in 10 samples is 0.30 pH. With one exception the values come within 0.05 pH of the average value.

The H correction, Austin, Stadie and Robinson (colorimetric pH 38°—electrometric pH 38°) of 41 samples gives an average value of 0.02 pH. The variations are from —0.07 to 0.11 pH. Twenty-two come within ±0.02 pH of the average value.

The average difference between the colorimetric value corrected to 20° and the colorimetric value at 38° of 54 samples is 0.21 pH. The variations are from 0.12 to 0.28 pH. Twenty-six come within ±0.02 pH of the average value.

Owing to the simplicity and rapidity with which colorimetric pH estimations may be made on blood plasma we have felt that this method should be saved, if possible. Although the cases studied do not include a great range of conditions, we believe there is a reasonably good agreement with the electrometric values. Details of this work will be reported shortly.

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[1] Myers, V. C., Schmitz, H. W., and Booher, L. E., *J. Biol. Chem.*, 1923, lvii, 209.

[2] Cullen, G. E., *J. Biol. Chem.*, 1922, lii, 501.

[3] Myers, V. C., *J. Biol. Chem.*, 1922, liv, 675.

[4] Hastings, A. B., and Sendroy, J., *J. Biol. Chem.*, 1924, lxi, 695.

[5] Austin, J. H., Stadie, W. C., and Robinson, H. W., *J. Biol. Chem.*, 1925, lxvi, 505.

3573

Some Effects of Ultra Violet Radiation on Hydra.

R. E. DEAL. (Introduced by W. W. Swingle.)

From the Zoological Laboratory, State University of Iowa, Iowa City.

In the following experiments an attempt has been made to determine the effects of ultra violet radiation upon Hydra, with respect to (1) physiological difference between species, (2) effects on budding, (3) effects of calcium chloride and magnesium chloride, (4) heat sensitization. All experiments were performed with a Pan Ray Arc lamp with an energy distribution of approximately 15 per cent ultra violet, 59 per cent visable, and 26 per cent infra red, with a total energy equivalent to 1 gm. cal. per sq. cm. per min. at 103 cm.[1] Each organism treated was exposed in a watch-glass containing five centimeters of pond water, placed sixty centimeters from the arc. The total energy of the arc at this distance is 2.94 gm. cal. per sq. cm. per min. The temperature for each experiment was determined by immersing the thermometer in the watch-glass. For the heat sensitization experiments the water in the watch-glass was kept at a temperature which varied no more than 0.5° C. by the use of an electric fan.

Experiment I. Physiological difference between species: (A) Three species of Hydra were used: (a) *H. viridis,* (b) *H. fusca,* (c) *H. dioecia.* Six series of 6 specimens each were employed for the series of tests, making a total of 108 Hydra. Series 1 was left in pond water at room temperature; series 2 was exposed to ultra violet for 15 minutes behind a glass screen 3 mm. in thickness; series 3 was placed in an electric oven until the temperature was raised from 18° C. to 28° C., a rise of 2° C. more than that which occurred during any ultra violet exposure; series 4 was exposed 5 minutes and series 5, 10 minutes to ultra violet; series 6 was exposed 15 minutes to ultra violet. Series 1, 2, and 3 showed no change, all specimens of the three species remaining normal. The results obtained from the exposures of series 4, 5, and 6 are as follows: (1) series 4, 8 hours after exposure all *H. viridis* and *H. fusca* were disintegrating, while 3 *H. dioecia* were dead and 3 disintegrating. All but three dioecia were regenerating after 48 hours. (2) Series 5, 3 hours after exposure all *H. viridis* and *H. fusca* were disintegrating, and 8 hours after all were dead. All *H. dioecia* were dead 3 hours after exposure. (3) Series 6, all *H. dioecia,* were dead immediately following exposure. Eight hours after exposure all *H. viridis* and *H. fusca* were dead.

(B) Groups of six each of each species, a total of 18 Hydra, were exposed 2 minutes daily to ultra violet for four days. All *H. viridis* and *H. fusca* were disintegrating after third exposure and all were dead 8 hours after last exposure. All *H. dioecia* were disintegrating after second exposure, 2 were dead after third exposure and all were dead after the last exposure.

It is evident that there is a physiological difference in reaction to ultra violet radiation in the three species of Hydra studied, and that *H. dioecia* is less resistant than either *H. viridis* or *H. fusca.*

Experiment II. Effects on budding: One series of 8 *H. dioecia* with buds was treated 10 minutes with ultra violet, and an additional series of 24 *H. dioecia* was exposed 5 minutes to ultra violet, a total of 32 Hydra. In the first series, 9 of the 12 buds were broken from the parent within 3 hours, and all showed partial disintegration. All the parents were dead within 3 hours. In the second series, every one of the 29 buds were broken from the parent within 5 hours, and all were alive after 48 hours. Eleven of the 24 parents were dead after 8 hours and the remaining 13 were regenerating after 48 hours.

The experiments indicate that there is a marked physiological difference between parent and bud of *H. dioecia* in their reaction to ultra violet radiation. The buds appear to be more resistant than the parents.

Experiment III. Effects of calcium chloride and magnesium chloride. Two series of *H. dioecia* with buds, each series containing 18 specimens, a total of 36, were exposed 5 minutes to ultra violet in a 1/8 of one per cent solution of calcium chloride and magnesium chloride, respectively. None of the buds were broken from the parent in either solution; 8 buds of each series disintegrated with the parent within 8 hours, and the remaining 10 were regenerating after 48 hours.

These experiments indicate that there is a chemical influence exerted by calcium chloride and magnesium chloride on budding Hydra. Further experiments of a similar nature are under way.

Experiment IV. Sensitization to heat: Two series of 10 specimens each, and one series of 20 specimens, a total of 40 Hydra, were exposed to ultra violet for varying periods at a temperature between 19° and 20° C. Two additional series of 20 specimens each, a total of 40 Hydra were exposed to ultra violet for varying periods at a temperature between 19° and 20° C., after which they were heated to a temperature of 28° C. in an electric oven. The results of these experiments show that in the first three series with exposures of 10, 5 and 3 minutes respectively, the per cent of deaths were 100, 50 and 35 respectively. In series 4 and 5 with exposures of 5 and 3 minutes respectively plus a rise in temperature, the per cent of deaths were 55 and 35 respectively.

These results indicate that exposure to ultra violet radiation does not induce sensitization to heat on the part of *H. dioecia*. This is not in accord with the results obtained by Bovie,[2] in which he found that Paramecia exposed to fluorite rays were sensitized to such an extent that they no longer were able to withstand their normal optimum temperature.

The lethal effects of ultra violet radiation on Hydra appear to be due to coagulation of the protoplasm.

This is a preliminary report.

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[1] Laurens, H., and Mayerson, H. S., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 506.

[2] Bovie, W. T., and Klein, A., *J. Gen. Physiol.*, 1918, i, 331.

3574

Sedimentation Rate of Erythrocytes and Plasma Proteins Following Prolonged Chloroform Anaesthesia in Dogs.

M. D. ROURKE AND E. D. PLASS.

From the Obstetrical Department, Henry Ford Hospital, Detroit.

Four dogs were anesthetized with chloroform for sufficient periods to insure liver necrosis. The sedimentation rate of the red blood cells, the albumin, globulin, and fibrin fractions from the plas-

TABLE I.
Dog No. 2. Male. Weight 14 lbs. Slight distemper. Chloroform given for 1¼ hours.

Sample No.	Time. Hours after anesthesia	Millimeters settled in 1 hour	Fibrin. Calculated from heparin hematocrit	Plasma.			Total protein	Hematocrit.		Remarks
				Fibrin	Albumin	Globulin		Heparin	Oxalate	
1	24.0	26.0	0.62	0.55	4.55	2.37	7.47	40.0	32.2	Plasma clear straw color
2	46.5	11.2	0.51	0.47	4.10	2.17	6.74	43.2	38.8	Plasma slightly yellow
3	68.5	8.0	0.16	0.14	3.70	2.52	6.36	43.6	36.5	Plasma bright yellow
4	81.0	1.3	0.12	0.11	3.76	2.43	6.30	43.6	38.5	Plasma and urine bright yellow
5	103.5	4.0	0.25	0.24	4.05	2.20	6.49	41.7	39.7	Plasma and urine bright yellow
6	129.5	10.0	0.34	0.33	4.07	2.24	6.64	40.0	38.0	Plasma and urine yellow
7	153.5	36.0*	0.42	0.41	— —	2.50	— —	35.7	34.5	Plasma slightly yellow
8	182.0	43.8*	0.61	0.59	3.46	2.74	6.79	31.0	29.8	Plasma clear straw color

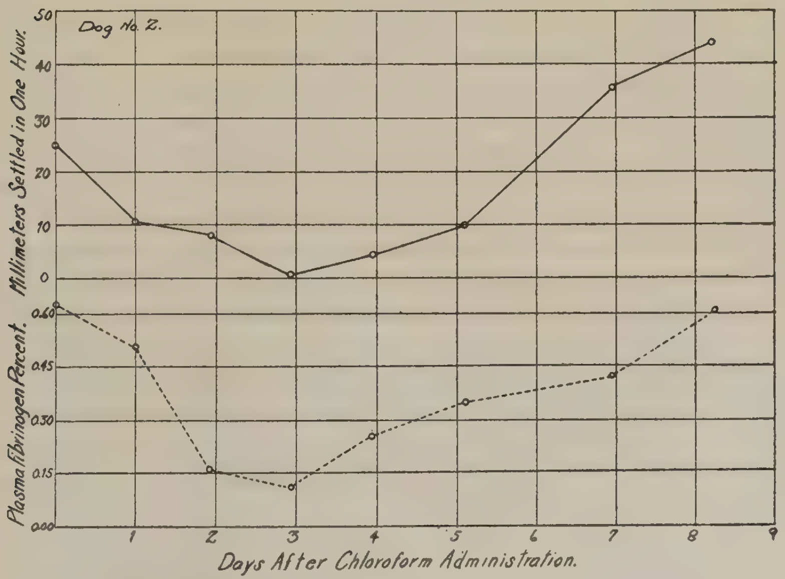
* Packing of the red blood cells was occurring, making these values somewhat lower than if the settling had not been impeded.

ma, and the hematocrit values were determined quantitatively.

The fibrinogen concentration in the plasma was considerably decreased during the height of the chloroform effect, but returned rapidly to normal during the recovery. Paralleling this drop in the fibrinogen, the sedimentation rate diminished, and later increased as the dog returned to normal. There was no significant variation in the albumin and globulin fractions. Relative increases in the bile pigments of the plasma and urine and of the lipoids of the plasma were noted.

The following table gives the data for Dog No. 2, characteristic of the series. In addition the chart given below shows graphically the relation between the rate of settling and the fibrinogen.

CHART 1.



These experiments tend to confirm the belief that the rate of sedimentation of the red blood cells is at least in part dependent on the fibrinogen content of the plasma.

This is a preliminary report.

Pacific Coast Branch.

Stanford University, Calif., April 30, 1927.

3575

Sulphate Reducing Bacteria in California Oil Waters.

RUDOLPH GAHL AND BELLE ANDERSON. (Introduced by T. D. Beckwith.)

From the Divisions of Bacteriology and of Biochemistry and Pharmacology of the Medical School of the University of California.

Edson S. Bastin has recently pointed out[1] that certain bacteria are responsible for the fact that oil well waters are low in sulphates and high in carbonates when compared with the sources from which these waters are presumably derived. He was able to isolate bacteria producing such chemical reactions from Illinois oil field waters. He also sampled oil-well waters originating from the Sunset-Midway and Coalinga districts in California. The samples were cultured by us and we were able to isolate from them sulphate reducing anaerobic bacteria of the same type as had been found in the Illinois oil field waters. Not all the wells, however, showed evidence of these organisms. The methods used for culturing will be described in another paper.

As the temperature and salt concentration range at which the organisms metabolize have been used by former investigators[2, 3, 4] to differentiate between various types, these influences were studied. All the organisms cultured were found to grow at room temperature and up to 37° C. Only some of them (4 out of 12) grew at 50° C. According to their preferences for certain salt concentrations the following groups were distinguished:

Type 1. Growth only in media without or with less than one per cent NaCl. Represented by 2 cultures only.

Type 2. Growth only in media containing NaCl. The organisms of this type seem also to be sensitive to high concentrations of salt, 3 per cent being the upper limit at which growth was obtained. This type is represented by 2 cultures only.

Type 3. Growth both in media with and without NaCl. Some of the cultures grew in all concentrations between 0 and 3 per cent salt, one culture between 0 and 5 per cent. In no case was the upper salt limit below 3 per cent. This is the most common type which we encountered.

The rule applies for all cultures that they either grow in a medium without or with 1 per cent salt, and it seems possible that there is a salt concentration somewhat below 1 per cent in which all of them grow. One is tempted to conclude that the organisms in the various wells developed from an ancestry living in somewhat less than 1 per cent salt and that the various descendants adapted themselves to varying conditions.

Dark field examination disclosed in every case the presence of a small motile vibrio. Staining classified the organism as Gram negative. It seems safe to conclude that this organism which coincides with the descriptions given by Beyerinck,[2] van Delden,[3] and Elion[4] of their sulphate reducing bacteria, morphologically identical with each other, is the causative agent of the sulphate reduction.

It is not possible to fit the organisms from the various wells into the groups suggested by the Dutch investigators previously quoted. If one wanted to stick to the Dutch classification one would have to assume for instance, that some of the wells contain a mixture of Beyerinck's desulfuricans and van Delden's aestuarii, both morphologically identical with each other.

It seems far more logical to assume that the various sulphate reducing vibrios represent the same organism which adapted itself to the conditions of various surroundings and thereby succeeded to live under more extreme conditions than those under which it was studied by the original investigator, Beyerinck. If this standpoint is taken, all the cultures studied appear as variations of the original vibrio desulfuricans.

We had an opportunity, through the courtesy of E. S. Bastin, to compare the living conditions of the bacteria in culture, so far as temperature and salt concentration are concerned, with the conditions which he had encountered, when he sampled the oil wells furnishing the bacteria. It is interesting to note that the bacteria which we had found able to live at 50° C. came from a definite small area of the oil field and that the temperature of these wells was the highest recorded, being nearly 50° C.

It is also worth noting that the organisms isolated from oil wells with low sodium chloride content refused to grow in media with as low as one per cent salt. There is, therefore, a close correspondence between the living condition observed in the field and the limiting conditions observed in the laboratory.

This is a preliminary report.

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[1] Bastin, Edson S., Anderson, Belle, Green, Frank E., Merritt, C. T., and Moulton, Gail, *Bull. Am. Assn. of Petroleum Geologists,* 1926, x, 1270-1299,

[2] Beyerinck, W. M., *Centr. für Bakter.*, etc., 1895, Abt. II, i, pp. 1-9 and 104-114.

[3] van Delden, A., *Centr. für Bakter.*, etc., 1903-4, Abt. II, xi, pp. 81-94 and 113-119.

[4] Elion, H., *Centr. für Bakter.*, etc., Abt. II, lxiii, 58-67.

3576

The Effect of Heparin on Anaphylactic Shock in Guinea Pigs.

O. B. WILLIAMS AND F. RENE VAN DE CARR. (Introduced by K. F. Meyer.)

From the Division of Bacteriology, University of California Medical School.

Kyes and Strauser[1] have recently reported the inhibition of anaphylactic shock in pigeons by the intravenous injection of heparin 45 minutes preceding the shocking dose of antigen. They state that it is their belief that many of the symptoms of anaphylactic shock are the result of the formation of fibrin aggregates, and that if the formation of these aggregates be inhibited by a suitable reagent the symptoms will be reduced or eliminated.

In view of the striking results of these workers with pigeons, it seemed to us that it would be worth while to determine the effect of heparin on anaphylactic shock in guinea pigs. The animals were sensitized to horse serum twenty days before testing. Eight controls were tested for efficiency of sensitization, 6 of these dying with classical antemortem symptoms and showing the typical post mortem picture. Two animals exhibited symptoms of severe shock but survived. The guinea pigs receiving heparin were given 5 mg. of heparin in physiological salt solution intracardially for each 100 grams of body weight. The results obtained with these animals are shown in Table I.

As shown in the table, two of the heparinized guinea pigs receiving shocking doses of serum exhibited classical symptoms of shock, one of them succumbing with typical post mortem findings. It may be recalled that Kyes and Strauser report that 1 of 12 heparinized pigeons showed transitory symptoms. We do not feel that there is any relation between the size of the shocking dose and the occurrence of severe symptoms in the heparinized guinea pigs, since some of the animals showed slight or no symptoms, after receiving the same amount of serum as that given to those which exhibited shock.

TABLE I.

Animal number	Time elapsing between injection of heparin and serum.	Amount of serum	Symptoms	Remarks
1	15 min.	2 cc.	None	
2	15 min.	2 cc.	None	Death 20 min. later from hemorrhage. No pulmonary emphysema. Pericardial sac filled with blood. Free blood in pleural cavity.
3	25 min.	2 cc.	Slight transitory respiratory distress	
4	30 min.	2 cc.	Typical symptoms of shock	Death in 5 min. with typical pulmonary emphysema at autopsy. Blood showed no clot in test tube after 48 hours.
5	40 min.	2 cc.	None	
6	55 min.	1.5 cc.	None	Death about 40 min. later from hemorrhage. Autopsy findings as for No. 2 above.
7	1 hour	1 cc.	Slight transitory respiratory distress	
8	1 hour	1 cc.	None	
9	1 hour and 15 min.	1 cc.	Slight transitory respiratory distress	
10	1 hour and 20 min.	2.25 cc.	Typical symptoms of shock	Recovered
11		None		Death from hemorrhage about 20 min. following heparin. Autopsy findings as for No. 2 and No. 6 above

A check on the clotting time of the blood of two of the control animals showed the formation of a clot at between 15 and 20 minutes, the blood having been collected in a test tube at autopsy. In the cases of the three heparinized animals which died from hemorrhage and of the one which died with symptoms of shock, no clot had been formed at the end of 48 hours by blood collected in a similar manner.

The animals for which we record slight transitory respiratory distress showed symptoms ranging from a single gasp to sneezing and scratching at the nose.

The results obtained appear to indicate that the injection of heparin into the circulation in amounts sufficient to prevent the formation of a blood clot prevents or reduces, in the majority of cases, the symptoms of anaphylactic shock in guinea pigs hypersensitive to horse serum.

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[1] Kyes, Preston and Strauser, E. R., *J. Immunol.*, 1926, xii, 419-422.

3577

Further Evidences of the Non-Sympathomimetic Action of Ephedrine.

FLOYD DE EDS AND EDWARD M. BUTT.

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In a previous report[1] it was shown that the most favorable functional state of the sympathetic nerves in blood vessels, *i. e.,* during sensitization by cocaine, failed to give responses to ephedrine, indicating that the cause of the pressor action of ephedrine was not sympathetic stimulation. Further evidence obtained recently along other lines confirms this result.

Three cats (4.5 to 7 cc. per kilo of Fledxt. Ergot, U. S. P.) and 1 dog (1 mgm. per Kg. ergotamine) were ergotized to the point of reversal of the original rises of blood pressure caused by epinephrine, to falls of pressure owing to paralysis of the constrictor sympathetic endings. In these same animals, the rises of blood pressure (before ergot) caused by different doses of ephedrine were invariably obtained when the vasomotor reversals to epinephrine were demonstrated. In other words, the pressor action of ephedrine still occurred after paralysis of the sympathetic constrictor endings, and

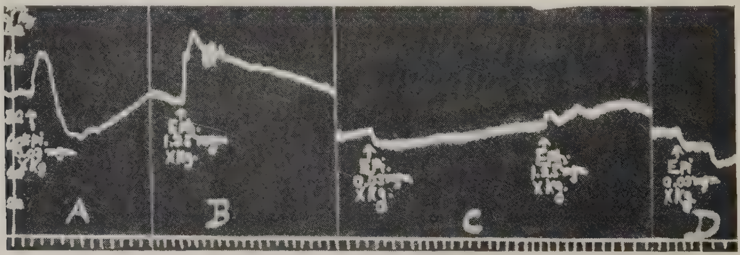

FIG. 1.
Control pressor actions of epinephrine and ephedrine (A and B), and reversal of epinephrine (C and D), but not with ephedrine (C) after ergot in a cat. "Epi" means epinephrine, and "Eph", ephedrine.

therefore, was due to muscular stimulation. Figures 1 and 2 illustrate typical results with different doses of ephedrine and epinephrine before and after ergot in a cat and a dog.

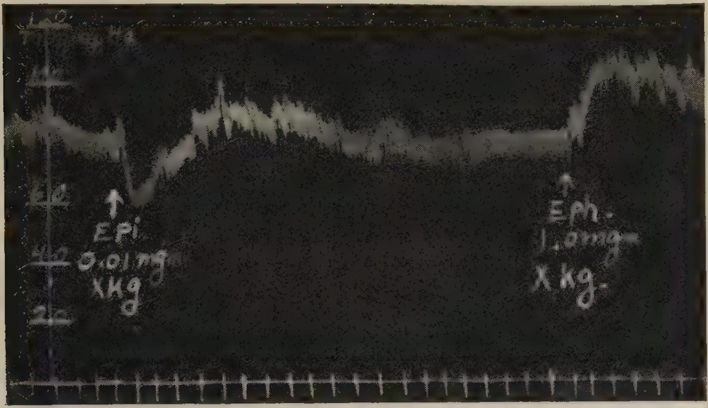

FIG. 2.
Pressor action of epinephrine reversed and pressor action of ephedrine preserved in an ergotinized dog; controls omitted, but were the same as usual.

Uteri responding by motor inhibition to treatment with epinephrine should respond similarly to ephedrine if the latter is sympathomimetic. However, this was not the case, for the same strips of excised uterus (in oxygenated Tyrode solution at 38°), which relaxed under epinephrine, were consistently contracted by the ephedrine. This biological test indicates that ephedrine stimulated the uterus through an action on the muscle. Figure 3 illustrates the contrast between epinephrine and ephedrine in their effects on excised guinea pig uterus. In a recent paper by Kreitmair,[2] appearing after this work was completed, the reported ephedrine stimulation of excised rabbit uterus, previously reversed with epinephrine with an ergot product, is consistent with our results. (See Fig. 3.)

Epinephrine had no action on the untreated bronchi of a decerebrated dog, previously atropinized and the vagus branches rendered non-responsive to electrical stimulation. But the bronchi were relaxed by ephedrine about as efficiently as by epinephrine when they were contracted by histamine and pilocarpine. This places ephedrine into the same category as epinephrie as a bronchodilator, the action not being sympathomimetic, but rather conditioned on the functional state of the bronchiolar musculature.

From all these results and the previous ones in cocainized animals, it is concluded that ephedrine* is not sympathomimetic in its actions on blood pressure, uterus and probably bronchi, the action being muscular. This and the greater chemical stability of the alkaloid suggest why its actions are better sustained than those of epinephrine.

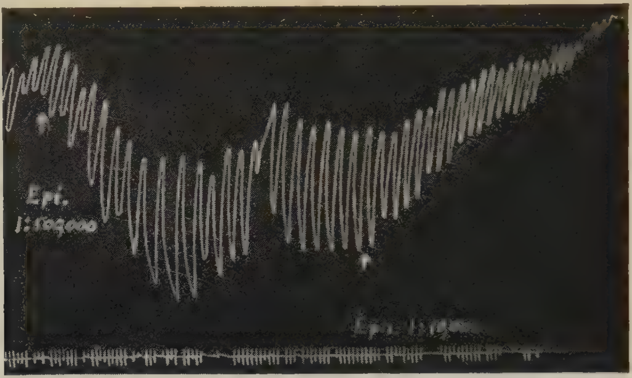

FIG. 3.
Strip of pregnant guinea pig uterus showing inhibitory response to epinephrine and augmentor response to ephedrine.

During the past two years different extracts and extractives (corresponding to alkaloid) of several specimens of *Ephedra nevadensis* obtained from Utah have been examined in this laboratory with negative results as to the presence of alkaloids and demonstrable physiological effects, except those which could be explained as due to the presence of tannin. Controls with extracts from Chinese ephedra and with ephedrine gave positive results.

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[1] De Eds, F., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 551.

[2] Kreitmair, H., *Arch. Exp. Path. Pharm.*, 1927, cxx, 189.

* The ephedrine used in this work came from two sources; a sample kindly supplied by Dr. Chen, and different samples marketed by the Abbott Laboratories.

3578

Studies in Tuberculosis. XI. Neutralization of Filterable Tubercle Toxin (T. F.) with Sheep Antiserum (Antitoxin).

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In continuation of the experiments reported previously,[1, 2, 3] Berkfeld filtrates from broth cultures of tubercle bacilli were used in the systematic immunization of a sheep. These toxin filtrates, hereafter designated as T. F., were obtained by growing a virulent strain of human tubercle bacilli in glycerinated "hormone broth" for 18 to 21 days and filtering the culture through a Berkfeld "N" candle. The sheep was injected subcutaneously at weekly intervals with increasing doses of freshly prepared filtrate. After four months, neutralizing properties of the serum were studied by the intracutaneous and intraperitoneal method in tuberculous and normal guinea pigs, and by skin tests in tuberculous and non-tuberculous human subjects.

Having found previously that an intracutaneous injection of unheated T. F. in the experimental tuberculous animal caused a definite skin reaction, the effect of an incubated mixture of immune sheep serum and T. F. was now studied.

Intracutaneous Method. Series of four guinea pigs each were infected with human tubercle bacilli, respectively 3 and 9 weeks and 3 and 5 months before these tests were made. Intracutaneous injections were given of a mixture of equal parts of serum and T. F., T. F. in a dilution of 1:2, immune serum alone, a mixture of normal sheep serum and T. F., and a mixture of immune rabbit serum and T. F. The dose, 0.05 cc., was injected after incubating all of these materials for one hour at 37.5° C. on a water bath. The results showed consistently that immune sheep serum (or immune rabbit serum) prevented the occurrence of positive skin tests, whereas normal sheep serum or normal rabbit serum failed to do so. Positive reactions were characterized by an area of redness and deep induration varying in extent from 0.5 x 0.5 to 1.5 x 1.5 cm. and lasting several days.

Intracutaneous Method. Series of four guinea pigs each were inproperty of antiserum from the sheep was obtained by injecting intraperitoneally mixtures of immune serum and T. F. incubated for 1 hour at 37.5° C. Tuberculous guinea pigs reacted uniformly to an intraperitoneal injection of from 1 to 2 cc. of T. F. with the exhibition of toxic symptoms that came on rapidly following a dose of the filtrate. Dyspnea, spasmodic contraction of the diaphragm simulating hiccough, marked weakness of the extremities, and profound prostration, lasting several hours, occurred regularly in all of the animals so tested. When from two to three-fold doses of T. F. were combined with immune sheep serum the typical reactions were prevented. Normal serum failed to accomplish this result and in normal guinea pigs the T. F. alone or in combination with immune or normal sheep serum produced no symptoms of intoxication.

Skin Tests, Adults. There were 180 patients with advanced tuberculosis, of whom 84 showed clinical improvement and the remainder did not. Intracutaneous tests were made with T. F. diluted 1:20 in physiological salt solution and incubated mixtures of equal parts of T. F. (diluted 1:10) and immune sheep serum. One-tenth cubic centimeter of the test material was injected into the skin.

Reactions were uniformly positive with T. F. in cases of advanced tuberculosis showing definite clinical improvement and were negative in patients who were failing and had a bad prognosis. In early tuberculosis more marked reactions were noted, and in apparently normal adults who were clinically non-tuberculous but had the usual amount of spontaneously healed tuberculous foci, skin tests were uniformly positive and intense. Immune serum combined with T. F. prevented the occurrence of skin reactions that were produced by one-half the amount of T. F. alone.

Children. This group included 60 patients, of whom 40 were definite contacts to tuberculosis and the remainder not exposed. Positive skin tests with 0.1 cc. of a 1:20 dilution of T. F. were observed in the group of contacts only. These children gave substantial evidence of tuberculosis in healed, arrested or active stages, and the severity of the skin reaction was correlated quite uniformly with the type of infection present. Tests with the ether-soluble tuberculin fraction in these children were negative. An additional series of 378 children, tested with tuberculin fractions, gave uniformly negative skin reactions with the ether-soluble derivative. From these

TABLE I. *Summary of Results.*

Group of Patients	Number	Skin Reactions		
		T. F. (dil. 1:20)	T. F. (dil. 1:5) plus equal volume of immune sheep serum	Ether soluble tuberculin fraction (dil. 1:50)
Tuberculosis (advanced)	180			
With clinical improvement	84	Positive	Negative*	
Failing, with bad prognosis	96	Negative	Negative*	
Clinically non-tuberculous adults with spontaneously healed foci	60	Positive	Negative	
''Contact'' children	378			Negative
	40	Positive	Negative	Negative
''Non-contact'' children	20	Negative	Negative	Negative

*16 patients tested; 13 with improvement—T. F. positive; 3 failing, with bad prognosis—T. F. negative.

results it may be deduced that the T. F. is not related to any possible bacillary substances most likely to give skin reactions. Of such elements the fatty derivatives, soluble in ether or acetone, are the ones to be controlled.

The skin tests made on adults and children and experimental animals suggest, furthermore, that T. F. offers a method for detecting existing tuberculous infection that is clinically significant, particularly in children. In this connection, ordinary tuberculin tests have not proved valuable.

The toxin filtrate of tubercle bacilli measures antitoxin content or susceptibility to tuberculous infection. A negative intracutaneous test with T. F. indicates either the absence of tuberculous foci and hence no resistance, or an advanced infection with profound toxemia and the amount of antibody entirely absent or too small for detection. In advanced tuberculosis, the reactions appeared to be correlated with the clinical condition of the patient and the prognosis in each case.

From these results and the studies previously noted, it may be concluded that the requirements for the demonstration of toxin by tubercle bacilli have been fulfilled.

Further studies are now in progress on methods of standardizing immune serum against toxin filtrates of different potency. Active immunization experiments have been begun with T. F. in animals and in children.

This is a preliminary report.

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[1] Eberson, F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 79.

[2] Eberson, F., *ibid.*, 1927, xxiv, 329.

[3] Eberson, F., *ibid.*, 1927, xxiv, 331.

SCIENTIFIC PROCEEDINGS.

Peking Branch.

Peking Union Medical College, April 28, 1927.

3579

Susceptibility of the Hamster to Tuberculous Infection.

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The white mouse is known to be extremely resistant to both the bovine and the human strains of the tubercle bacillus. Koch found the field mouse more susceptible than the white mouse, but it is not known with what species of field mouse he worked. Certainly is was not the striped hamster, which is not found in Central Europe.

The striped hamster *(Cricetulus griseus)* is very susceptible to infection with *Leishmania donovani* and these micro-organisms seem to mobilize the same type of cell that is mainly concerned in phagocytizing tubercle bacilli and initiating tubercle formation. So it was but natural to wonder if the hamster is not susceptible to tuberculous infection. The experiments here cited show this to be the case.

Known bovine and human strains of tubercle bacilli have been introduced by the subcutaneous, intra-abdominal and oral routes. The guinea pig was used chiefly to test the pathogenicity of the strains employed, as it is known to react to infection in a more or less uniform way. In the subcutaneous and intra-abdominal inoculations, homogeneous suspensions of 0.1 mgm., dried weight, of tubercle bacilli were administered to hamsters and white mice, and 1.0 mgm. to guinea pigs, as the weight of the latter was approximately ten times that of the former. In the feeding experiments an excess of tubercle bacilli was mixed with the feed, no attempt being made to measure the amount ingested. One animal of each series was sacrificed weekly. At autopsy gross studies were made, and microscopic examination of sections of lung, liver, spleen and tracheo-bronchial lymph nodes was done, and it was found that after six weeks of infection a gross diagnosis of tuberculosis of the lungs could be made in nearly every hamster, whereas this could not be done in the majority of the white mice. The gross lesions in the hamster were not so large as in the guinea pig and showed less tendency to caseate, but tubercle bacilli were much more numerous in the hamster's tissues and usually could be found easily in smears from the lungs, tracheo-bronchial lymph nodes and spleen. In the feeding experiments sections from the intestines were studied. In 4 out of 10 hamsters that had been infected with the human strain

TABLE I.
Incidence of Tuberculous Infection of Hamsters and Guinea Pigs from Subcutaneous Inoculation of Tonsils and Pleural Fluids.

Case No.	Inoculation	Hamster.			Guinea Pig.		
		Amt.	Gr.	Mic.	Amt.	Gr.	Mic.
1	L. T.	0.4	0		4.0	0	0
	R. T.	0.4	0	0	4.0	0	0
2	L. T.	0.4	+	+	4.0	+	+
	R. T.	0.4	+	+	4.0	+	+
3	L. T.	0.2	0	0	2.0	0	0
	R. T.	0.4	0	0	4.0	0	0
4	L. T.	0.1	0	0	1.0	0	0
	R. T.	0.1	0	0	1.0	0	0
5	P. F.	1.0	0	0	4.0	+†	+†
	P. F.	0.5c*	0	0	4.0c*	0	+†
6	P. F.	1.0c	0	0	4.0c	0	0
	P. F.	1.0	0	0	4.0	0	0
7	P. F.	1.0c	0	0	4.0c	0	0
	P. F.	1.0	0	0	4.0	0	0
8	P. F.	1.0c	+	+	4.0c	+	+
	P. F.	1.0c	+		4.0c	+	†
9	P. F.	1.0	+		4.0	+	+
10	P. F.	1.0	0	0	4.0	0	0
	P. F.	1.0c	0	0	4.0c	0	0
11	P. F.	1.0c	+	+	14.	0	0‡

*Centrifugalized sediment. † and ‡ see text.

for more than 5 weeks the duodenal wall showed tubercles; in 4 out of 10 infected with the bovine strain the duodenal wall likewise showed tubercles and the wall of the colon showed them in three. The white mice were all negative. Apparently the hamster develops generalized tuberculosis through ingestion as readily as does the guinea pig. Yet, in 200 careful autopsies, we have not seen spontaneous tuberculosis in the hamster. At autopsy the spleen was weighed routinely and was found to have increased markedly in the infected hamsters but only slightly in the white mice.

The dosage was large, and it might be wondered whether the hamster is sufficiently susceptible to be useful as a diagnostic medium for tuberculosis in the hospital. Table I illustrates a parallelism between the hamster and the guinea pig in susceptibility to tuberculous infection, even when relatively small numbers of bacilli are introduced. The tonsils in Case 2 did not show tuberculosis histologically, though serial sections were not made. None of the pleural fluids showed tubercle bacilli when examined microscopically. In Case 11 the hamster showed tuberculosis while the guinea pig did not. The latter was inoculated intraperitoneally. There is another discrepancy in Case 5 where the hamsters are negative and the guinea pigs positive. A plausible explanation lies in the fact that the second guinea pig of Case 8, which was in the same cage with those of Case 5, died on the nineteenth day after inoculation and was found largely eaten by the guinea pigs of Case 5. As the former was tuberculous the latter could have become infected in this way. Elsewhere[1] the more pratical usefulness of the hamster for this type of diagnostic work in China is discussed.

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[1] Korns, J. H., *China Medical Journal.* In press.

3580

On Carbohydrate-Like Specific Substance in the Colon Aerogenes Group.

JOSEPH TOMCSIK. (Introduced by Carl TenBroeck.)

From the Department of Pathology, Peking Union Medical College.

Specifically reacting polysaccharides have been isolated by various authors from pneumococci, pneumobacilli, tubercle bacilli and yeasts. Preliminary unpublished experiments convinced us that substances with varying chemical composition and carbohydrate content could be obtained easily and in large quantities from different encapsulated bacilli (*B. coli, B. anthracis* and encapsulated soil bacteria). *B. lactis aerogenes* was chosen in the present study, since in the early literature Emmerling[1] and Schardinger[2] succeeded in isolating a nitrogen free polysaccharide from the filtrated cultures of these organisms.

The strain chiefly used for the preparation of the polysaccharide was isolated from human feces. Its behavior in sugar media was typical, acid was produced on citrate agar, the indol reaction was negative and the Voges-Proskauer test positive. Capsule formation was distinct though not marked.

Following the method of Emmerling and Schardinger an attempt was made to isolate a carbohydrate from the filtrate of a synthetic fluid medium culture. The yield of specific substance was small and failed to cause precipitation in dilutions higher than 1:10,000 when layered over homologous immune serum. It was found, that even after 30 days incubation the bulk of the specific substance was associated with the bacterial bodies which still showed a small, but distinct capsule.

The method finally adopted was essentially the same as that described by Toeniessen.[3] At first the bacilli were cultivated on a synthetic medium solidified with agar containing ammonium phosphate as a source of nitrogen, and sodium citrate as the carbon source. Among many substances tried, the addition of small amounts of prophyl-red more than doubled the growth. In later work meat infusion agar was used with equally satisfactory results. The two days old growth from 100 Kolle flasks, suspended in distilled water and precipitated with alcohol, yielded 11 gm. of dried bacteria. This could be resuspended evenly in 1800 cc. distilled water. The capsules of the bacteria were found to be intact when the suspension was examined in Indian ink or stained with Hiss capsule stain. After the addition of 200 cc. of 100 per cent KOH, the suspension was placed in the incubator (37° C.), and every half hour samples were taken out for the examination for capsules. These usually disappeared at the end of 3 or 4 hours and at this time the solution was separated from the bacteria by centrifuging. To the supernatant fluid acetic acid was added until no more precipitate (nucleoproteid) came down, after which the fluid was passed through a Seitz filter. Three times the volume of alcohol was added to the clear filtrate and 2.1 gm. white powder obtained. This powder was easily soluble in water or saline and gave specific precipitation with the corresponding immunserum when diluted 1:200,000. It contained 1.4 per cent nitrogen.

With this procedure 5 gm. material was collected for further purification. The alcohol precipitation was repeated six times in the presence of n/10 sodium acetate in slight alkalic reaction. The precipitin titer of the resulting powder was 1:300,000 (faint reaction in 1:1,000,000). The nitrogen determined both with Nesslerisation and according to the micro Kjeldahl procedure titrating with 0.005/n iodometric solutions was still 0.9 per cent. A 1:200 solution gave negative Biuret and Millon tests, was not precipitated after boiling with acetic acid or after the addition of trichloracetic acid, silver-nitrate, or Fehling solution. It gave partial precipitate with barium-sulphate and neutral lead acetate, and both the precipitate and the supernatant contained the specific substance. It was precipitated quantitatively by the addition of alkalic lead acetate, phosphotungstic acid and colloidal ferrihydroxide. With Benedict's reagent there was no reduction. After hydrolysis with n/H_2SO_4 (Optimum time 4 to 5 hours at 100° C.) its reducing substance counted as glucose corresponds to 66 per cent.

Further purification was attempted by absorption with aluminiumhydroxide. In this we followed the technic of Heidelberger, Goebel and Avery,[4] but instead of "A" grade aluminium hydroxide, we used "B" grade, which seemed to us easier to prepare and according to Willstätter highly effective in the purification of enzymes. Practically the same specific titer and nitrogen content was found after the absorption. Uranyl nitrate could not be used for further purification as it caused no precipitation.

Specific substances were prepared from five other strains of aerogenes with the same cultural and biological characteristics. None of these substances reacted specifically with the first immunserum. With one of these strains immunserum was produced in rabbits, which was precipitated by the homologous specific substance, in a dilution of 1:100,000 but again gave no reaction with the substances obtained from the other strains. The strict specificity of these substances makes it difficult to attempt any typing by this means in such a heterologous group of organisms.

A similar substance isolated from encapsulated *B. coli* reacted also in very high dilution with homologous immune sera and showed the same narrow type specificity.

To determine whether these substances were antigenic, rabbits were injected in the same way as with the bacterial culture, but with considerably larger quantities of dry material. Precipitations were not produced.

Protein free hapten was isolated from a strain of *B. lactis aerogenes*. This substance consists chiefly of polysaccharides, but in spite of several attempts at further purification, its nitrogen content could not be reduced below 0.9 per cent. Similar substances obtained from several other aerogenes strains and from encapsulated *B. coli* showed a strict individual specificity, no cross reactions were observed.

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[1] Emmerling, O., *Ber. d.d. chem. Ges.*, 1900, xxxiii, 2477.

[2] Schardinger, F., *Cent. f. Bakt.*, Abt. II, 1902, viii, 144.

[3] Toeniessen, E., *Cent. f. Bakt.*, Orig., 1920-21, lxxxv, 225.

[4] Heidelberger, M., Goebel, W. F., Avery, O. T., *J. Exp. Med.*, 1925, xlii, 701.

3581

Anaphylactic Shock Produced by a Soluble Specific Substance Largely Carbohydrate in Nature.

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In order to study the relation of the precipitin test to anaphylaxis, and to determine whether a substance largely carbohydrate in nature and failing to give protein reactions would produce shock, the following experiments were made. The aerogenes specific substance described in the previous paper, containing 0.9 per cent N and giving a precipitate with immune serum when diluted 1:500,000, was used for the tests.

Twenty guinea pigs were sensitized by the intraperitoneal injection of 1 cc. aerogenes immune serum, and the response of their uteri tested, using the Schultz-Dale method. The uteri of all these animals reacted to the specific substance when tested from 2 hours to

12 days after the injection of serum. The smallest amount of specific substance that caused a distinct but not maximal contraction, was 0.00000625 gm. added to a bath of 123 cc. so that the final concentration of the substance was 1:20,000,000. Desensitization of the uteri was demonstrated after the contraction due to one addition of 0.005 gm. of the substance to the bath.

When tested for sensitiveness to specific substances obtained from other strains of aerogenes there was no response, so that the results correspond to those obtained with the precipitin reaction.

As controls, the uteri from 6 normal guinea pigs were tested and not one gave a reaction following the addition of as much as 0.01 gm. of specific substance to the bath.

In vivo tests were made on 11 guinea pigs 24 hours after the intraperitoneal injection of from 1 to 4 cc. immune serum. Ten of these animals died showing the typical symptoms and signs of anaphylaxis. The M. L. D. of the specific substance for these sensitized animals was 0.000033 gm. The animal receiving 0.00002 gm., showed symptoms, but survived. Control tests on untreated animals show that 0.001 gm. failed to cause any reaction.

Attempts to sensitize guinea pigs with the specific substance alone were negative.

In this, which is a preliminary report, it is shown that in passively sensitized guinea pigs anaphylactic shock is produced by very high dilutions of a substance that is largely carbohydrate in nature. The presence of the small amount of nitrogen, presumably as an impurity, prevents us from concluding definitely that shock may be produced by carbohydrate alone. Further work with specific substances from other organisms is in progress.

3582

Optimal Proportions of Hapten and Immunserum in the Precipitation and Complement Fixation Reactions

JOSEPH TOMCSIK. (Introduced by Carl TenBroeck.)

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In a recent paper Dean and Webb[1] have shown that just as in the Ramon test there is an optimum zone in mixtures of protein and its antiserum where precipitation occurs more quickly, and that the indicator or first tube in which a precipitate appears contains neither protein or antiserum in excess. They also find that after the reaction is completed the largest amount of precipitate is not found in this indicator tube. Dean's[2] previous work has shown that a mixture that gives the maximum precipitate is not the one that fixes the largest amount of complement.

TABLE I. *Precipitation.*

Antiserum dilution	Time of reading in minutes	Dilution of the yeast gum.													
		3,000	4,000	6,000	8,000	12,000	16,000	24,000	32,000	48,000	64,000	96,000	128,000	192,000	256,000
		Grade of the turbidity* and flocculation.													
1:5	5	3	3	5	5	6	6	5	5	3	2	2	1	1	–
	12	4	4	6	7	±	7	6	5	3	2	2	2	2	1
	30	4	4	+	++	++	++	++	++	+	4	4	3	2	2
1:10	5	–	–	1	2	3	4	5	5	4	3	2	2	1	–
	30	–	1	2	3	4	5	+	6	5	5	4	2	2	2
	40	1	2	4	5	6	±	+	+	±	6	5	3	3	2
	90	4	6	7	7	±	+	++	++	+	+	±	±	5	5
1:20	5	–	–	–	–	–	1	2	3	3	3	2	2	2	1
	30	–	–	–	–	1	2	3	4	5	4	3	3	2	2
	60	–	–	1	2	4	5	6	6	7	6	6	4	3	3
	120	–	1	2	3	4	6	6	±	+	+	±	6	5	4

*The grade of the turbidity is given in arbitrary numbers from 1 to 7 and the amount of flocculation indicated by +.

Our object has been to study the quantitative relationship of antigen and antiserum in both the precipitin and complement fixation reactions in order to determine whether they are related. We have

TABLE II. *Complement Fixation.*

Antiserum dilution	Incubation time in minutes	Temperature	Units of complement	Dilution of the Yeast Gum.												
				24,000	32,000	48,000	64,000	96,000	128,000	192,000	256,000	384,000	512,000	768,000	1,024,000	1,536,000
1:20	1	room	20	++	+++	++++	+++	±	—	—	—	—	—	—	—	—
1:40	1	room	10	—	+	+	+++	+++	+++	+	—	—	—	—	—	—
1:80	1	room	2	—	—	±	+	+	+	++	+	+	±	—	—	—
1:160	10	37°	2	—	—	—	—	—	±	++	+++	++++	+++	+++	++	±
1:320	15	37°	2	—	—	—	—	—	—	±	+	++	++	++	++	±

considered throughout the first tube giving precipitate or fixation so that our method differs from previous work where emphasis was placed on the maximum precipitate. Because of their great sensitivity we have used carbohydrate haptens rather than proteins as antigens. One of these haptens is a carbohydrate gum obtained from a pathogenic yeast, and the other the aerogenes specific substance described in the preceding paper. Both of these gave a precipitate with their respective immune serum when diluted 1:500,000 and complement fixation when diluted 1:64,000,000.

For the precipitation reaction the method of Dean and Webb was followed. Dilutions lower than 1:5 and higher than 1:40 could not be used, as in the former precipitate occurred at once in several tubes, and in the latter there was a slight opacity only.

It is difficult to restrict the fixation of complement to a few tubes, but by using varying amounts of complement and different incubation times and temperatures it is possible to determine for each dilution of serum the zone where maximum fixation takes place. Thus when the serum was diluted only 1:20 it was necessary to use 20 units of complement and to add the hemolytic system 1 minute after the complement.

In tables I and II are given the results obtained from tests made with the various dilutions of immune serum. It should be noted that for these tables we have selected the experiments where the narrowest zones of precipitation or complement fixation were obtained. It will be seen that when the dilution of antiserum is doubled, the dilution of hapten in the indicator tube is also doubled. Comparison of the two tables shows that approximately the same ratio of antiserum to antigen, 1:2400, in the indicator tube holds for both the precipitin and complement fixation tests in the various dilutions used.

Similar results showing that with serogenes specific substance the ratio of antiserum to antigen in the indicator tube is approximately the same in both precipitin and complement fixation reaction will be reported later in detail.

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[1] Dean, H. R., and Webb, R. A., *J. Path. and Bact.*, 1926, xxix, 473.

[2] Dean, H. R., *Zeit. f. Immun.*, 1912, xiii, 84.

3583

The Partition of Serum Calcium into Diffusible and Non-Diffusible Portions.

SHIH-HAO LIU. (Introduced by F. R. Dieuaide.)

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Various methods of determining the diffusible and non-diffusible calcium have given discordant results. The question whether the non-diffusible calcium is protein-bound is unsatisfactorily answered. The present communication gives, first, the relative importance of the two fractions of blood calcium in maintaining the normal irritability of neuro-muscular system, second, the correlation between non-diffusible calcium and the protein content of blood or other body fluids, and third, the influence of cod liver oil and parathyroid hormone on the calcium partition in blood.

For the determination of diffusible and non-diffusible calcium, the method of ultra-filtration and dialysis as described by Moritz[1] and improved by Updegraff, Greenberg and Clark[2] was employed with a few modifications. Total calcium, diffused calcium and non-diffused calcium were separately analyzed, using the method of Clark and Collip.[3] Wu and Ling's method[4] was used for albumin and globulin content of blood or other body fluids.

The calcium distribution in blood serum was determined in 9 adults with conditions not known to be associated with disturbances in calcium metabolism, with a total serum calcium ranging from 9.2 to 10.6 mg. per 100 cc. The non-diffusible calcium varied from 6.1 to 4.1 mg. or 57.6 to 42.3 per cent, while the diffusible calcium from 5.6 to 4.5 mg. or 57.7 to 42.4 per cent. The average values of 5.0 mg. or 51 per cent for diffusible calcium, and of 4.8 mg. or 49 per cent for non-diffusible calcium obtained in this series agree very well with the results of Moritz and Updegraff and her co-workers.

Two cases of tetany were studied. In one case with a total serum calcium of 8.0 mg., the non-diffusible fraction was 4.3 mg. or 58.6 per cent, while the diffusible fraction 3.7 mg. or 46.4 per cent. In the second case with a total serum calcium of 6.5 mg., the results were 3.8 mg. or 56.4 per cent for the non-diffusible portion and 2.6 mg. or 43.6 per cent for the diffusible portion. In these two cases of tetany, both the diffusible and the non-diffusible calcium was decreased with a greater decrease in the diffusible fraction.

In the three cases of nephrosis examined, all showed a reduction of total serum calcium, the values being 8.6 mg., 7.9 mg. and 7.7 mg. respectively. The non-diffusible fraction was greatly reduced, namely, 3.3 mg., 2.3 mg. and 2.0 mg., while the diffusible fraction remained normal or slightly above normal, namely, 5.3 mg., 5.6 mg. and 5.7 mg. Similar results were obtained in 4 cases of kala-azar studied. The total serum calcium ranged from 6.6 mg. to 7.6 mg. In spite of the low total serum calcium in these cases of kala-azar and nephrosis, none of them showed any increased irritability of nerves to either mechanical or electrical stimulation. The greater reduction of diffusible than of non-diffusible calcium in tetany, and the reduction of the non-diffusible portion without any reduction in diffusible calcium in kala-azar and nephrosis without tetany, suggest strongly that it is the diffusible, rather than the non-diffusible calcium that is physiologically active in the control of irritability of neuro-muscular system.

The reduction of non-diffusible calcium in nephrosis and kala-azar seems to be associated with a reduction in plasma protein, especially albumin. In these cases, plasma albumin was invariably low, ranging from 1.2 to 2.3 per cent with an average of 1.9 per cent, in contrast to 3.7 per cent in the 9 control cases. Plasma globulin was distinctly low in four out of seven cases. Studies on 8 pleural and ascitic fluids seem to substantiate further the close parallelism between non-diffusible calcium and protein content. In these fluids, with a total calcium ranging from 5.3 to 7.2 mg., the non-diffusible portion varied from 1.7 to 0.2 mg., while the diffusible ranged from 5.7 to 4.4 mg., showing that almost all the calcium is in the diffusible fraction. The protein content of these fluids was extremely low; albumin 0.4 to 0.06 per cent, and globulin 0.7 to 0.04 per cent.

The influence of cod liver oil on serum calcium partition and plasma protein was studied in two cases. One case of tetany with a total serum calcium of 8.0 mg., consisting of 4.3 mg. of non-diffusible and 3.7 mg. of diffusible calcium was given 30 cc. of cod liver oil daily for 12 days. At the end of the period, the total serum calcium rose to 9.8 mg., 4.7 mg. non-diffusible and 6.1 mg. diffusible, showing a more marked rise in the diffusible calcium. Similar results were obtained in a case of nephrosis. In these cases the plasma proteins were not markedly changed, although the albumin showed a tendency to increase toward the end of the period.

Next, the effect of parathyroid hormone was studied in two cases of tetany. In one case, which was already convalesceent, 730 units of Collip's parathyroid extract were given in 8 days. The serum calcium rose from 9.2 mg. to 10.4 mg. In regard to the calcium partition, the non-diffusible portion increased from 4.2 mg. to 4.6 mg., while the diffusible portion from 5.2 mg. to 5.8 mg. The second case, while showing active tetany, received 530 units of the extract in 8 days. The blood calcium increased from 6.5 to 11.1 mg. The non-diffusible fraction changed from 3.1 to 4.6 mg., while the diffusible fraction rose from 3.6 to 6.6 mg. In both cases, under the influence of parathyroid hormone the rise in the diffusible calcium was greater than in the non-diffusible calcium. Plasma proteins again were not significantly altered, though the tendency for the albumin to diminish was appreciable.

To summarize, in the study of serum calcium, emphasis should be laid on its partition, for there are conditions unassociated with tetany but showing a low total serum calcium. In such cases, unlike those of tetany, the reduction is entirely in the non-diffusible calcium, coinciding with a reduction of plasma albumin. The importance of protein in relation to the level of non-diffusible calcium is again brought out by the analysis of pleural and ascitic fluids which with a very low protein content contain mostly diffusible calcium, the non-diffusible being sometimes negligible in amount. Under the influence of either cod liver oil or parathyroid hormone, the elevation of blood calcium seems to be more in the diffusible than in the non-diffusible portion, apparently independently of any change in the plasma proteins. The increase of the diffusible calcium in the cases of tetany is always accompanied by relief of symptoms.

A full report will be published in the *Chinese Journal of Physiology*, July, 1927.

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[1] Moritz, A. R., *J. Biol. Chem.*, 1925, lxiv, 81.

[2] Updegraff, H., Greenberg, D. M., and Clark, G. W., *J. Biol. Chem.*, 1926, lxxi, 87.

[3] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, lxiii, 461.

[4] Wu, H., and Ling, S. M., *Chinese J. Physiol.*, 1927, i, 161.

3584

The Alleged Ephedrine Action of Two California Species of Ephedra.

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In a recent paper by Clark and Groff[1] it was inferred that ephedrine or closely related substances are present in two California species of Ephedra. By blood pressure experiments with rabbits a 10 per cent infusion of *Ephedra nevadensis Wats,* was shown to have a marked effect in causing large rises of blood pressure. Other experiments with *Ephedra californica Wats,* indicated that it also contained a pressor material.

Samples of these two varieties of Ephedra were obtained from Dr. Walter Swingle, Department of Agriculture, Washington, and an attempt was made to isolate ephedrine from them. Using similar amounts and identical procedures of extraction there were prepared solutions of the active principles from these two species, and from Peking Ephedra, purchased in local drug stores. The chloroformic extracts were submitted to standard methods of assay, tested with

TABLE I.

Tests of alkaloidal residues from three Ephedras which were assayed by identical procedures.

	Peking Ephedra	*E. californica*	*E. nevadensis*
Titration with N/10 HCl	67.80 cc.	0.82 cc.	2.35 cc.
Equivalent percentage of Ephedrine	1.12 per cent	0.014 per cent	0.04 per cent
Nagai's test with alkaline $CuSO_4$	Very deep violet	No color	Very faint tinge
Nessler's solution	Bulky ppt.	No change	*Very* faint reaction
Average rise in B. P.	32 mm.	0	0.5 mm.

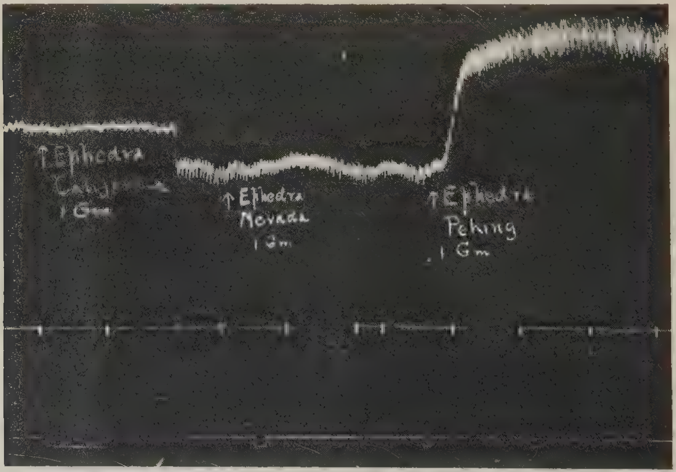

FIG. 1.

Blood pressure tracing of extracts of three Ephedras injected into a male dog, 8.7 kilos, anesthetized with luminal.

alkaloidal reagents and finally compared by Nagai's alkaline copper sulphate test. Then these extracts were used for physiological testing. Three intravenous injections of each of the two Californian Ephedras before and after an identical injection of Peking Ephedra were made with a large dog, suitably anesthetized and canulated for blood pressure tracing.

The chemical assay and testing, as compared with known extracts of specimens of Peking Ephedra containing ephedrine, showed absolutely no ephedrine in our sample of *E. californica, Wats,* nor was there anything more than possibly an exceedingly small trace in *E. nevadensis, Wats.* Both species gave no rise in blood pressure as did the preparations from Peking Ephedra. These results lead to the inference that the results formerly obtained with the infusions of Ephedra were probably due to the presence of simple colloids, and certainly not to basic substances related to ephedrine, unless there be exceedingly great seasonal variation in the alkaloidal content of this plant.

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[1] Clark, G. W., and Groff, G. W., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 325.

3585

Blood of Animals in Hematoporphyrin Shock.

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Experiments designed to throw light on the state of the blood of animals sensitized by hematoporphyrin and exposed to light were carried out on cats, guinea pigs, rats and mice.

One to 2 cc. per 100 gm. of body-weight of a 0.5 per cent solution of crystalline hematoporphyrin hydrochloride (Nencki) in n/10 NaoH or 1 to 2 per cent $NaHCO_3$ were injected subcutaneously, intraperitoneally, or intravenously. Sunlight was usually employed. Normal animals and animals similarly injected but kept in the dark were studied throughout as controls.

In preliminary observations no difficulty was encountered in securing the usual reaction of such sensitized animals on exposure of the skin to light.

Exposure of the peritoneum alone was followed by shock, but exposure of blood flowing through a glass canula inserted in the femoral artery or between the femoral artery and femoral vein produced shock in only 1 of 5 cases.

Mice fed on hematoporphyrin by mouth for 8 weeks did not develop any sensitization to light.

Blood Pressure. The carotid pressure of cats sensitized by hematoporphyrin and exposed to light falls from normal values of 120 to 160 mm. Hg. to 30 to 50 mm. within 35 to 120 minutes. The rapidity of fall depends upon the intensity of the light. A prompt reaction to adrenalin is maintained. The heart beat and the respiration cease simultaneously.

Pulse Rate. Both pulse and respiration rate increase markedly during the development of shock and are slowed towards the end of the experiment.

Blood Cells. The number of red blood cells remains practically unchanged; during the early stages a leucocytosis of 13,000 to 18,000 appears, which is followed by a leucopenia of 4,000 to 5,000.

Blood Chemistry. The amount of sugar, total non-protein nitrogen and creatinine was found to be essentially the same as in control animals.

The oxygen content of mixed venous blood from the right ventricle of guinea pigs in hematroporphyrin shock falls from 11 to 14 volumes per cent to 6 to 10 volumes per cent, while the oxygen capacity remains unchanged.

The carbon dioxide combining power of the serum falls from 37 to 54 volumes per cent to 16 to 20 volumes per cent in spite of the introduction of 10 to 15 cc. of 1 to 2 per cent $NaHCO_3$ in which the hematoporphyrin was dissolved.

Toxicity of the Blood. From 0.5 to 1.5 cc. of the heart's blood of guinea pigs in hematoporphyrin shock was injected into mice and produced no effect different from that of the injection of the same amount of heart's blood from control animals.

In a parabiosis of 2 rats hematoporphyrin shock induced in one was not communicated to the other.

Effect of Light on Sensitized Blood in vitro. Sensitized blood exposed *in vitro* to light changes its color to dark reddish brown. The resistance of the red blood cells to hypotonic saline solutions falls and hemolysis occurs.

Gas analysis shows changes similar to those found *in vivo,* decrease in the oxygen content without change in the capacity, and increase of the carbon dioxide content.

3586

Kala Azar Transmission Experiments With Chinese Sandflies (Phlebotomus).

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We reported[1] the development of herpetomonad flagellates in Chinese sandflies fed on hamsters infected with kala azar. In the present paper are summarized the results of various kala azar transmission experiments performed in 1925 and 1926.

The three species of sandflies, for the determination of which we are indebted to Professor R. Newstead, are: *Phlebotomus major* var. *chinensis* Newstead; *P. sergenti* var. Newstead *(Phlebotomus "C"* of our previous report), identical with an unnamed variety described from Mesopotamia[2]; *P. perturbans* var. Patton[3] *(Phlebotomus "B").* This latter species we have never fed on man or other mammals in the laboratory, but it readily engorges on toads.

In the following experiments we tested the hypothesis that *Phlebotomus* sandflies becoming infected by sucking the blood of kala azar patients are then able to transmit the disease to other persons by their bites. The first was fed upon heavily infected hamsters. Later they were fed upon tested negative hamsters.

As *P. sergenti* became infected with *Leishmania* only rarely, following "natural" feeds, as contrasted with the very large proportion of infected *P. major,* a technique described elsewhere[4] was devised for feeding sandflies artificially with rich suspensions of Leishman-Donovan bodies. The apparatus consists essentially of a cork vise for immobilizing the sandfly and an adjustable holder for the feeding pipette, the end of which is fitted over the insect's stylets. Freshly defibrinated rabbit blood was centrifuged and the serum removed. Spleen tissue from a hamster heavily infected with Leishmania was ground up in this serum. A volume of spleen-serum suspension equal to that of the serum, originally removed, was then added to the rabbit corpuscles. The sandflies engorged readily. Infections with Leishmania were produced in all three species. Those for *P. perturbans* are not reported here, since feeding experiments on mammals have not proved possible with this species.

All the sandflies were laboratory-bred. The items are classified according to the number of days after the first, or potentially in-

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fective, blood meal of the sandflies concerned. "Positive" and "negative" sandflies indicate the results of examinations for Leishmania either in fresh preparations, stained smears or in sections. Hamsters bitten by positive sandflies represent tested negative striped hamsters from which at least one sandfly, demonstrated at dissection to contain Leishmania, is known to have sucked blood. Hamsters "exposed" are those cases in which no blood could be demonstrated in the sandflies after the feeding cages were removed. As visible blood was the criterion of feeding the number of hamsters classified as bitten represents actually the minimum subjected to the bites of potentially infected sandflies. Our routine was to keep the hamsters for 6 months after the experiment to allow ample time for an infection to become demonstrable. The animals were then killed with ether, and smears of the spleen, liver, bone marrow and heart-blood were examined microscopically. Negative findings in hamsters "dead within 90 days" are considered inconclusive and these are therefore indicated in a separate column. A number of hamsters injected intraperitoneally with positive sandflies developed heavy visceral infections of Leishmania (41 out of 180, or 23 per cent) thus demonstrating the pathogenicity of the organisms present in the sandflies, *but the entire series of experiments involving 413 hamsters bitten by or exposed to the bites of infected or potentially infected sandflies of two species without exception has resulted negatively.** Certain marked differences between *major* and *sergenti* are clearly shown. *Major* refeeds with reluctance, while *sergenti* refeeds readily. *Major* is very susceptible to infection with Leishmania following natural feeds (195 positive out of 348, or 56 per cent). Artificial feeds yield a particularly high proportion of infections (131 out of 157, or 83 per cent). The infection in *major* tends to persist. On the other hand, *sergenti* following natural feeds becomes infected only rarely (16 out of 661, or 2.4 per cent). The massive doses in the artificial feedings yield a greatly increased proportion of infections (169 out of 349, or 48 per cent), but in both naturally and artificially fed *sergenti* there is a tendency for the infection to die out after the fifth or sixth day.

In addition to hamsters injected with positive sandflies, 44 hamsters were injected intraperitoneally with 27 lots, aggregating 96 *sergenti* females, potentially infected but negative at dissection. No infections developed in any of the hamsters.

Five hamsters were inoculated by scarification with two *major* females containing flagellates of Leishmania. The hamsters were negative at autopsy.

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* Completely negative results from a comparable feeding series have been obtained with *P. argentipes* by Shortt, Barraud and Craighead. *Ind. J. Med. Res.*, 1927, xiv, 589.

260 female *sergenti,* taken mostly in kala azar houses were inoculated intraperitoneally into 53 hamsters. Control suspension smears made for each sandfly were all negative; 183 additional females, of which no control smears were made, were inoculated into 24 hamsters. Nine of the 77 hamsters died within 90 days. No infection developed in either these or the 68 survivors. 118 females and 48 males examined by smears only were negative. A lot of 353 females and 41 males of *sergenti* from the same group of houses were all negative. Thus a total of 904 females and 89 males of *sergenti* from a kala azar region examined by smears or by inoculation into hamsters or by both methods, have been negative for Leishmania. About 200 other sandflies, including both *major* and *sergenti,* were all negative for flagellates. A large proportion of *perturbans* were heavily infected with a crithidiform flagellates. No infection developed in 32 hamsters inoculated intraperitoneally with 124 such wild *perturbans.*

Sergenti is the dominant sandfly in kala azar villages near Hsüchowfu but is infected with difficulty and such infections tend to die out. *Major,* on the other hand, while easily infected may be very plentiful in places where there is little or no kala azar. Neither species has been infected following feeds on kala azar patients, nor have infections with Leishmania been found in wild sandflies.

Summary. Although sandflies infected with Leishmania may produce infections in hamsters when inoculated intraperitoneally, all efforts to transmit the infection to hamsters by their bites have failed.

This is a preliminary communication.

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[1] Young, C. W., and Hertig, M., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 611.

[2] Newstead, R., *Bull. Ent. Res.,* 1913-14, iv, 309.

[3] Patton, W. S., and Hindle, E., *Proc. Roy. Soc., Ser. B,* 1926, c, 405.

[4] Hertig, A. T., and Hertig, M., *Science,* 1927, lxv, 328.

Illinois Branch.

Northwestern University Medical School, May 10, 1927.

3587

Symptoms and Fiber Degenerations Following Experimental Lesions in Subthalamic Nucleus of Luys.

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This study is based upon 5 dogs with a lesion in the subthalamic region, in which the subthalamic nucleus of Luys was particularly involved. Two lesions were made with a probe, the remaining 3 by the injection of a very weak solution of silver nitrate. The animals were allowed to live from 8 to 11 days, and their symptoms observed. The brains were then prepared by the Marchi method for degenerated nerve fibers.

A large fasciculus of very fine rather diffusely scattered fibers leave the subthalamic nucleus on its medial side, the ventral part of the fasciculus being embedded in the cerebral peduncle. These fibers then run caudad as a wide band over the dorsal and medial surface of the *substantia nigra* and are distributed as follows:

(a) About half of these subthalamic fibers terminate in a nucleus of large, rather closely packed cells occupying an area in the form of an inverted comma. The broad, somewhat rounded head of this nucleus is located between the medial side of the *substantia nigra* and the mammillary peduncle. From this head a wedge-shaped group of cells extends laterally over the dorsal surface of the *substantia nigra*. This group of cells begins caudal to the mammillary bodies and extends beyond the upper level of the red nucleus.

(b) Many of the fibers from the subthalamic nucleus of Luys pass more medial and terminate in a field of more scattered cells near the midline, dorsal and medial to the mammillary peduncle, and within the tegmentum ventral and medial to the red nucleus. This field of cells begins just cephalad to the red nucleus and continue almost to its caudal level.

(c) A considerable number of subthalamic fibers cross the midline caudal to the mammillary bodies and terminate on the opposite side in the same manner as the homolateral fasciculus.

The most pronounced symptom following a lesion in the subthalamic nucleus of Luys was a hypertonic condition of the muscles. This varied in degree from a mild hypertonicity to a marked spasticity according to the extent of the lesion. The hypertonicity was usually most pronounced on the side opposite the lesion, affected the muscles of the legs more than those of the body and the hind leg more than the front. The hypertonicity was accompanied by an awkwardness in handling the corresponding muscles. The movements were slow, deliberate, halting and inaccurate, lacking the rhythm of a normal movement.

The animals showed an increase in temperature of from 2 to 3 degrees, and a rate of heart beat which was 15 to 45 beats more than normal. There was a partial recovery from all these symptoms within a few days after the operation.

This is a preliminary report.

3588

Conditions Affecting the Emptying-Time of the Human Gall-Bladder.

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One of the authors reported the occurrence of sex differences in the contraction rate of the human gall-bladder.[1] Since then ten additional cases have been examined which, with the others, seem to establish the fact that the average emptying time of the gall-bladder in young women is considerably quicker than in young men. But as yet no explanation of this has been forthcoming; nor, indeed, of individual differences between members of the same sex.

Believing that gall-bladder action might be correlated with the rate at which a meal moves through the intestinal tract, a number of these individuals were given a barium meal and its course followed for several days.* In addition, the contents of the fasting stomach were titrated, following which the patient was given a standard Ewald meal. The changing acidity of the stomach was then determined by the fractional method and the results plotted against the contraction curve of the gall-bladder (Fig. 1).

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* The gastro-intestinal examination was made under the direction and through the generous cooperation of Professor Hartung of the Department of Roentgenology.

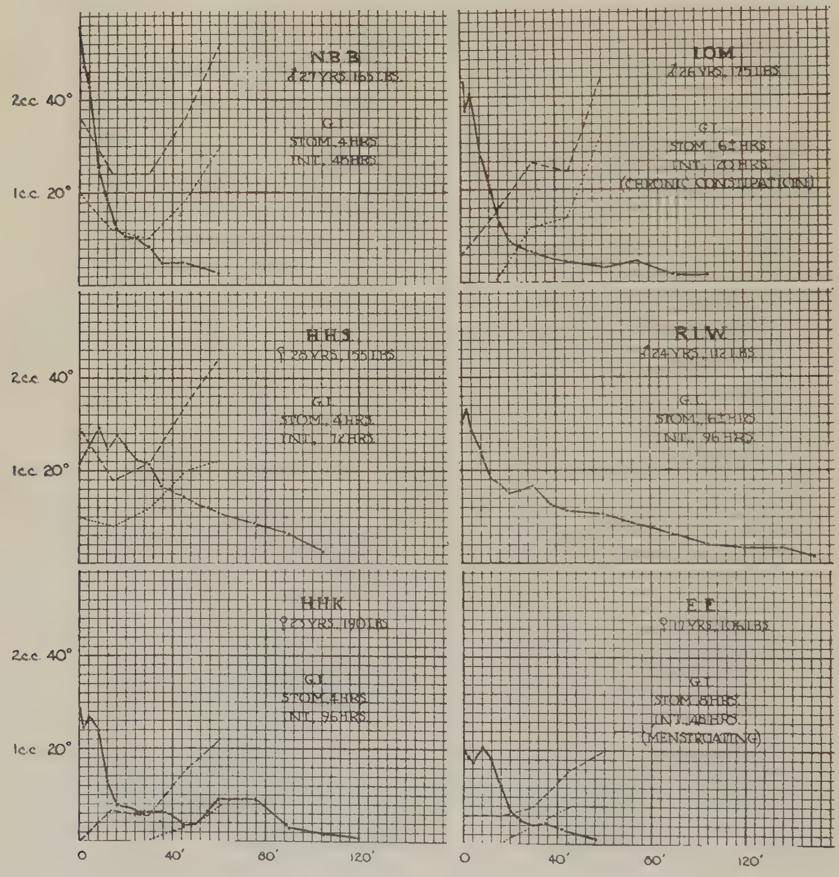

FIG. 1.

Comparing emptying time of gall-bladder with gastric acidity and with the motility of the stomach and intestine. CC., cubic inches of bile in gall-bladder; 40′, 80′, 120′, minutes after a meal; 20°, 40°, degrees of gastric acidity; dot and dash line, contraction curve of gall bladder after standard meal of egg yolk and cream (dots indicate successive x-rays); dash line, total acidity; dotted line, free acid in stomach following standard Ewald meal; STOM., emptying time of stomach (barium meal); INT., length of time required to pass barium meal through the intestine. R. L. W. In this individual two attempts were made to titrate the gastric contents, but in neither instance could the stomach tube be retained.

The primary object in testing the stomach was to eliminate from the series any individuals with gastric ulcer or carcinoma, gastritis, pernicious anemia and nervous disorders. But no such conditions were found, so that the stomach of these individuals may be considered normal. Although it was not expected that the amount of free acid present in the fasting stomach could influence the gall-bladder, since the meal of egg yolk (by which the action of the gall-bladder was tested) would probably neutralize the acid, it is possible that some of the gastric juice might have been pushed ahead of the meal by the first increment of egg yolk, which (as viewed under the fluoroscope) passes immediately into the duodenum. If such is the case no correlation was found to exist between the amount of acid in the fasting stomach and the emptying of the gall-bladder. Thus the gastric acidity of N. B. B. (Fig. 1) whose gall-bladder emptied very fast, is virtually the same as that of H. H. S., whose gall-bladder emptied very slowly (*cf.* also case N. B. B., who had a high initial acidity, with L. O. M., who had no free acid in the fasting stomach). Nor could any correlation be established between the emptying time of the stomach and of the gall-bladder, for in the three cases at the left of Fig. 1 the stomach contents were evacuated in four hours, while the gall-bladder behaved very differently in the three individuals. An even more striking contrast appears in cases E. E. and H. H. K., in which the gall-bladders reacted the same, but the emptying time of the stomachs was markedly different.

Again, a comparison of the six cases in Fig. 1 reveals a lack of correlation between the gall-bladder and the motility of the intestinal tract. The most striking case is that of L. O. M., who had chronic constipation associated with an anomalous loop of the colon, 120 hours being required to move a barium meal through the intestine. Yet this individual was one of the two cases (out of 12 males) in which the gall-bladder emptied the fastest.

Of special interest is case E. E., in which the gall-bladder was tested during the first day of menstruation. Nevertheless the rate of emptying seems not to differ from that of H. H. K. and others who were examined during the intermenstrual period. The smelling of food has no effect upon the emptying of the gall-bladder, and gravity is not a retarding factor since the contraction curve of an individual x-rayed when reclining may be duplicated several months later, when the same person is x-rayed in a standing position.

A theory has been advanced which attributes the expulsion of bladder bile to the milking action of peristalsis.[2] But Whitaker has shown that egg yolk and barium may pass through the intestinal tract of a cat without affecting the gall-bladder.[3] Recently, a human case showed little if any response for six hours, during which time two fatty meals were eaten and a bowel movement recorded. The next day, following other meals the gall-bladder was nearly emptied. It is, therefore, questionable whether peristalsis plays any rôle in emptying the biliary reservoir. This is confirmed by the following experiment (Fig. 2) designed to eliminate all expulsive factors but the *tunica muscularis* of the *vesica fellea.*

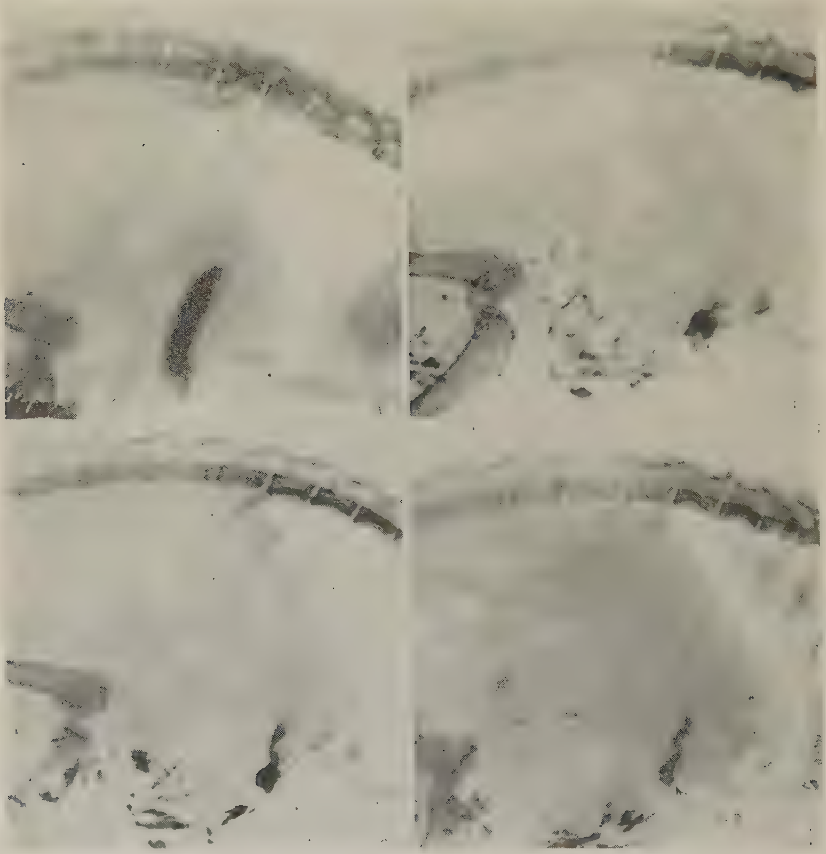

FIG. 2.

The gall-bladder of a cat was filled with lipiodol and the common bile duct severed just above the point where it entered the duodenum (the intestinal end being tied). Before the abdomen was closed lipiodol was observed to be emerging from the cut end of the duct. Three hours later an x-ray was taken (Fig. 2a), the cat being then in good condition, Little if any bile had entered the peritoneal cavity notwithstanding the struggle accompanying the emergence of the animal from ether anesthesia. By the next morning much of the contents of the gall-bladder had been emptied spontaneously (as frequently happens during fasting[4]), proving that the orifice of the bile duct had not been closed by adhesions. At this time the gall-bladder was relaxed (G. B., fig. 2b). Eight minutes after feeding egg yolk, however, the gall-bladder elongated, as if under tonus (2c), and in the course of the next few hours expelled additional amounts of lipiodol into the cavity (2d). But in no case was the purported sucking action of intestinal peristalsis operative, since the bile duct had been severed from the intestine.

In conclusion, one must therefore assume that the mechanical passage of food through the intestinal tract is not a factor in the emptying of the gall-bladder.

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[1] Boyden, E. A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 353-358.

[2] Burget, G. E., *Am. J. Physiol.*, 1925, lxxiv, 583.

[3] Whitaker, L. R., *Am. J. Physiol.*, 1926, lxxviii, 418.

[4] Boyden, E. A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 157-162.

3589

The Importance of the Carbonate Ion in Physiological Activity.

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From the Department of Physiological Chem. and Pharmacology, University of Chicago.

In the course of a study of the response of the guinea pig uterus to pituitrin when the ionic environment of the uterus was varied, the carbonate ion was found to be of importance in determining the magnitude of the response. The carbonate ion seems to exert its influence of itself, and not to be merely reflecting a change in calcium or hydrogen ion activity. This is shown by the following experimental results.

Whenever the carbonate ion activity was increased there was a diminished response of the uterus to pituitrin. This was true whether the carbonate ion activity was increased (a) by increasing the bicarbonate activity and CO_2 tension proportionately so that there was no change in pH; (b) by increasing the bicarbonate activity alone with an increase in pH; or (c) by decreasing the CO_2 tension alone, which also resulted in an increased pH.

Whenever the carbonate ion activity was decreased there was an increased response of the uterus to pituitrin. This occurred whether the carbonate ion activity was decreased (a) by decreasing the bicarbonate ion activity and CO_2 tension proportionately without change in pH; (b) by decreasing the bicarbonate without change in CO_2 tension thereby lowering the pH; or (c) by increasing only the CO_2 tension which also resulted in a decreased pH.

When the carbonate ion activity was kept as constant as experimental technique permitted, there was either no change in response or a slight change which might at times be positive and at other times negative. This occurred regardless of whether the bicarbonate was doubled, the CO_2 tension quadrupled and the resulting pH decreased, or the bicarbonate was halved, the CO_2 tension quartered and the resulting pH increased.

The calcium ion activity was the same in all solutions.

3590

Influence of "Effective Temperature" Upon Bactericidal Action of Gastro-Intestinal Tract.

LLOYD ARNOLD AND LOUIS BRODY.

From the Department of Bacteriology and Department of Pediatrics, University of Illinois.

We have previously shown the effects of acid and alkaline buffered substances fed by mouth upon the bactericidal action of the gastro-intestinal tract.[1] We explained these differences upon the variations in the H-ion concentration of the material in the upper half of the small intestine. When there was a predominance of acid or acid buffered substances in the lumen of this part of the tract, there was an auto-disinfection of the exogenous bacterial flora. When there was a predominance of alkaline buffered substances in the upper part of the small intestine, there was a loss of this power to kill bacteria.

We wished to use certain methods of changing in external environment to influence gastro-intestinal function and study the same bactericidal mechanism under these conditions. Bread was soaked in cooked meat broth and fed to dogs kept in rooms at ordinary and warmer temperatures. Prodigiosus was added to the food before feeding. Temperature in the cool room was 50° F., the relative humidity was 40 per cent. The warm room was 98° F. and 70 per cent relative humidity. The "effective temperature" in this instance was 91°.[2] We have used several effective temperature zones, but for brevity wish to report a typical middle zone of effective temperature in this paper. Each dog reacted differently in maintaining his internal temperature. Some dogs even in the above temperature environments did not have a rise in rectal temperature, while others had a rise of 4° F. Drinking water was always kept in the room for the dogs.

TABLE I.

The per cent of bacteria fed by mouth appearing in the caecum in relation to the time of ingestion. Four dogs at cool and warm room temperatures.

	Cool Room				Warm Room			
Time after feeding	A	D	Br	Bl	A	D	Br	Bl
0	0	0	0	0	0	0	0	0
½ hr.	0	0	0	0	0	0	50%	0
1 hr.	0	0	0	0	0	0	100%	0
1½ hrs.	0	0	0	5%	0	15%	75	0
2 hrs.	0	0	0	30%	5%	25	75	0
2½ hrs.	0	0	0	30%	5%	40	100	75
3 hrs.	0	0	15%	0	10%	10	100	75
4 hrs.	0	0	0	0	40	25	100	75
5 hrs.	0	0	20%	0	25	0	100	75
6 hrs.	0	0	0	0	30	0	100	75
7 hrs.	0	0	30%	0	25	0	100	50

Table I gives the results of a typical experiment illustrating differences in the bacterial killing power of gastro-intestinal tract of the same dogs upon the same diet in the cool and warm rooms.

This is a preliminary report.

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[1] Arnold, L., and Brody, L., *Am. J. Hygiene*, 1926, vi, 672.

[2] Houghten, F. C., and Yagloglou, C. P., *J. Am. Soc. Heat. and Vent. Eng.*, 1923, xxix, 165, 515.

3591

Susceptibility of Gastro-Intestinal Tract to Irritating Action of Salmonella Group of Food-Poisoning Bacteria.

LLOYD ARNOLD AND CHARLOTTE SINGER.

From the Department of Bacteriology, University of Illinois.

There is a bactericidal mechanism in the small intestine that causes the destruction of bacteria injected directly into the duodenum, or that pass through the stomach into the intestine.[1, 2, 3, 4] Savage and

White[5] have shown that certain types of "food-poisonings" can be caused by heat-stable toxic substances in *B. enteritidis* infected food material. Young dogs (3 to 6 months old) were fed meat and bread. The meat was ground up and mixed with equal amount of 1 per cent NaCl solution, autoclaved. Some samples were mixed with bread to proper consistency and fed as controls. *B. enteritidis* (5 strains obtained from Prof. E. O. Jordan) was inoculated in some of these meat flasks, incubated 24 hours, and stood at room temperature for 5 to 7 days before being mixed with bread and fed to the dogs. In the third experiment, the same *B. enteritidis* infected meat was placed in the boiling water for 45 minutes, bread added and fed as in other experiments. All food was warmed to 30° to 35° C. before feeding.

Cool or ordinary temperature room was 50° F. and 40 per cent

TABLE I.

Gastric acidity of dogs 2½ hours after feeding meat and bread in cool and warm rooms.

	Cool Room Temp. 50°, H. 40% *Acidity*		Warm Room Temp. 98°, H. 70% *Acidity*	
	Free	Combined	Free	Combined
Plain meat and bread 12 young dogs	0	120	0	57
Enteritidis infected meat and bread 14 young dogs	0	95	0	35
Heated enteritidis infected meat and bread 18 young dogs	0	90	0	18

TABLE II.

Distribution of *B. prodigiosus* through gastro-intestinal in percentage of amount fed to dogs.

Meat with bread	Before feeding	Stomach	Duodenum	Upper jejunum	Lower jejunum	Ileum	Cecum
T. 50, H. 40 Plain	100%	0	0	0	0	0	0
T. 50, H. 40 Enteritidis	100%	0	0	0	0	10%	10%
T. 50, H. 40 Heated enteritidis	100%	0	0	0	25%	35%	35%
T. 98, H. 70 Plain	100%	15%	15%	25%	35%	100%	100%
T. 98, H. 70 Enteritidis	100%	15%	25%	25%	50%	100%	100%
T. 98, H. 70 Heated enteritidis	100%	100%	100%	100%	100%	100%	100%

relative humidity. Warm room was 98° F. and 70 per cent relative humidity; effective temperature was 91°. Windows and doors in both rooms were closed. Table I gives the results of gastric analysis on 44 dogs fed with the three above mentioned mixtures. These were acute experiments, dogs anesthetized 2½ hours after feeding.

Table II shows the relative distribution of *B. prodigiosus* in the gastro-intestinal tract 2½ hours after ingestion under the same conditions as the previous experiment. Six young dogs 4 months old of the same litter were used. Relation distribution is expressed in percentage of the original dose of the chromogenic bacteria.[3] All of the dogs fed heated *B. enteritidis* infected meat in the hot room vomited one or more times and acted as if they were sick. A few of the dogs given the unheated living *B. enteritidis* infected meat vomited. This was not the rule and the amount vomited was always small. The cool room dogs never vomited with any of the above food mixtures.

We have used a small number of kittens (6) and have found the same changes as have been described for the young dogs. Average temperature of dogs was 101.2, in warm room. There was an average elevation of rectal temperature to 104° F.

These results confirm our previous findings and substantiate the experimental work reported by Savage and his colleagues. There is a toxic substance present in the *B. enteritides* infected meat that acts as a gastric irritant and causes a diminution in the secretion of acid. This substance is heat stable and as Savage[5] reports, the toxicity is increased by heating. This is accompanied by an inhibition of the normal bactericidal mechanism of the intestinal tract.

This is a preliminary report.

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[1] Arnold, L., *J. Inf. Dis.*, 1926.

[2] Arnold, L., and Brody, L., *J. Inf. Dis.*, 1926.

[3] Arnold, L., and Brody, L., *Am. J. Hyg.*, 1926.

[4] Arnold, L., *Klin. Wochschr.*, 1927.

[5] Savage, W. G., and White, P. B., *Med. Res. Council Spl. Rep.*, No. 92, 1925.

3592

B. Melitensis and B. Abortus Agglutinins in Dispensary Patients.

LLOYD ARNOLD AND W. E. MILLER.

From the Department of Bacteriology, University of Illinois College of Medicine.

Sera obtained from routine Wassermann test were tested for agglutinins, for *B. melitensis* and *B. abortus* in the average outdoor dispensary population in Chicago. *Brucella melitensis* variety *Melitensis* (A) No. 428, and variety *abortus* No. 456 were sent to us by Alice C. Evans and were used as our antigen. The technic recommended by Evans[1] was rigidly carried out. Up to the present, 200 sera have been tested; dilutions have been 1:10, 1:20, 1:40, 1:80, and 1:160. Fourteen sera have been positive within this range of dilutions. All positives were retested and found in each case to check with the previous tests.

Table I gives our results.

TABLE I.

	Antigen	
Serum No.	*B. melitensis*	*B. abortus*
1.	1:40	1:20
2.	1:20	1:20
3.	1:10	1:20
4.	1:40	1:40
5.	1:40	1:20
6.	1:20	1:20
7.	1:10	1:10
8.	1:40	1:40
9.	1:20	1:20
10.	1:40	1:20
11.	1:10	1:20
12.	1:10	1:10
13.	1:10	1:20
14.	1:20	1:40

Evans[2] has reviewed the literature. She found 58 sera in 500 from patients suffering with a variety of diseases to be positive in dilutions of 1:5 to 1:40, and suggests that these were produced as a specific response to *Br. melitensis* ingested in cow's milk, although such an infection may not necessarily have caused a notable illness. Three of our positives in 1:40 dilution were normal pregnant women. The others were in a variety of non-febrile cases. A milk-drinking history can always be ascertained from a patient. If the explanation of the occurrence of these agglutinins in the serum offered by Evans is correct, we have found so far 7.0 per cent of the average dispensary population have a specific response to *B. melitensis* and *B. abortus*.

This is a preliminary report.

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[1] Francis, E., and Evans, Alice C., *U. S. Pub. Health Reports*, 1926, xli, 1273.

[2] Evans, Alice C., *U. S. Pub. Health Reports*, 1924, xxxix, 501.

3593

On Bile Stimulation of Pancreatic Secretion.

A. C. IVY and H. C. LUETH.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Mellanby[1] has found that the injection of bile of an adequate reaction into the duodenum of the cat stimulates the pancreas. This also occurs after ligation of the pylorus and bile ducts, and after atropine and ergotamine. He suggests that bile is the alimentary stimulus of pancreatic secretion, functioning by causing the elaboration and absorption of secretin. However, in Mellanby's experiments bile may have caused stimulation by a local nervous mechanism that is not acted on by atropine or ergotamine.

Our work has been confined entirely to dogs. We have found that bile by stomach tube stimulates pancreatic secretion, but not invariably and to the extent that N/10 HCl does. Two dogs with a pancreatic fistula and the common bile duct doubly ligated, have responded to a meal of ground meat (half lean and half fat) as well as pancreatic fistula dogs without the bile duct ligated. Two dogs with a pancreatic transplant reponded as well to a meal of meat after double ligation of the bile duct as they did before the ligation. Bile, when applied to the Thiry fistula of a pancreatic-transplant-Thiry-fistula preparation, stimulates the transplant occasionally, but not uniformly. Bile applied to a pancreatic-transplant-jejunal-transplant preparation does not stimulate, whereas N/10 and N/20 HCl do.

Our observations cause us to conclude (1) that bile stimulates pancreatic secretion, but is not as potent as N/20 HCl, and (2) that bile is an adjuvant, but not an essential alimentary stimulus of pancreatic secretion in the dog. This is an abstract.

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[1] Mellanby, *J. Physiol.*, 1926, lxi, 419; *Lancet*, 1926, ccxi, 215.

3594

Dye Excretion by the Pancreas.

E. OLDBERG AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

With a view of perfecting some test whereby the degree of the external secretory response of the pancreas may be accurately measured in man, we have attempted to determine the permeability of pancreatic acinus tissues to certain dyes intravenously administered.

We have used dogs, in which the main pancreatic duct has been cannulated and connected to a stoppered rubber bag by means of a Rehfuss tube. In some of our preparations, we have in addition ligated the lesser pancreatic duct, the common bile duct, or both. With such a fistula the pancreas secrete copiously. Our procedure has been to stimulate secretion by means of food or water, and then to inject intravenously from 30 mg. to 60 mg. of dye, depending upon its density, dissolved in 25 cc. of physiological salt solution. The continuous secretion has subsequently been followed at 15 minute intervals for periods of an hour or more, and then at irregular intervals for twenty-four hours.

Thus far we have run one or more injections of some 32 dyes, namely, Acid blue, Acid orange, Acid violet, Alizarine blue S., Alkali blue 6B, Aniline blue, Bieberich's scarlet, Bluish eosin, China blue, Congo red, Erythrosin, Hofmann's violet, Indigo carmine, Mendola's blue, Methylene azure I, Methylene blue, Methylene violet, Neutral red, Neutral violet, Orange G crystals, Orange I, Patent blue, Phloxine, Poirrier's blue, Pyrrol blue, Rose Bengal, Thymol sulfonphthalein, Toluidine blue, Tropeolin O, Tropeolin 000 No. 1, Tropeolin 000 No. 2, Trypan blue.

Of these, only two have appeared in the external pancreatic secretion. A heavy dose of methylene blue gives a faint bluish tinge to the secretion at the end of two hours; and a rather heavy dose of methylene violet colors the secretion a distinct reddish violet in from 2 to 3 hours. Methylene violet is toxic in large doses.

Although the pancreatic acinus appears to be a very selective secreting unit, we propose to extend our observations until we have a more complete knowledge of its permeability to other dyes.

This is a preliminary report.

3595

Time of Onset of Vagal Function in the Heart of Mammals.

HOWARD B. KELLOGG. (Introduced by A. C. Ivy.)
From the Department of Anatomy, Northwestern University.

Electrical stimulation of the peripheral end of the right vagus in ten pig fetuses which were about three-fourths term did not cause inhibition of the heart. Stimulation of the peripheral ends of the right and left vagus nerves of rabbits and dogs ranging from one hour to five days after birth caused no cardiac inhibition in 20 per cent of 32 animals examined; very weak inhibition was found in 36 per cent, and strong inhibition in 44 per cent. One rabbit and one dog showed a strong cardiac inhibition on stimulation of the sino-auricular node, even though stimulation of the vagi caused no such effect. It was very noticeable that in a large number of cases where inhibition was obtained it resulted from a vagal heart block, in which the atrial beat was increased in rate and frequently in amplitude. Stimulation of the central end of the vagus nerves inhibited respiration in all of the rabbits and dogs.

Apparently the afferent respiratory fibers of the vagus nerves are functional at birth, but in a large percentage of animals the cardio-inhibitor fibers either react weakly or are non-functional. There is some indication that the post ganglionic mechanism of the vagus in the heart may be functional before the preganglionic fibers are capable of transmitting impulses.

3596

Precipitation of Proteolytic Enzymes of Bacillus Proteus by Azine and Azonium Bases.

ARTHUR W. WALKER. (Introduced by A. C. Ivy.)
From the Department of Bacteriology, Northwestern University Medical School.

That there is a definite chemical union between a proteolytic enzyme and an azine or azonum base was first suggested by Robertson,[1] who observed that if a drop of a saturated solution of safranine was added to a solution of trypsin a flocculent precipitate was formed which gradually settled to the bottom of the tube.

Holzberg[2] tested the proteolytic activity of this safranine-trypsin precipitate and found that it hydrolized casein, thus proving that the enzyme was precipitated by the safranine. Marston[3] made a study of the chemical nature of this compound. He found that the proteolytic enzyme trypsin was completely precipitated from its solution by safranine, and also by several other compounds which were water soluble and contained the azine nucleus, such as neutral violet, neutral red and the more complex indulines.

Marston states that the type of union between the protease and the azine base in a direct combination of the enzyme with the azine nitrogen, the acid nature or property of the enzyme being responsible for this combination. As evidence of this union he says that with the reduced leuko compound of safranine where the nitrogen bonds are all satisfied with hydrogen, no precipitation of the proteolytic enzyme takes place. The results of the work quoted above suggested the possibility of purifying, and studying the chemical nature and action of bacterial proteases.

With this in view a series of experiments were begun. *Bacillus proteus* was selected as the organism to use as it excretes a very active proteolytic enzyme. A culture of this organism was inoculated into sugar-free nutrient broth, and incubated for five days. At the end of this period the broth culture was filtered through Berkefeld filters to remove the organisms. The clear filtrate consisting of the broth, plus the soluble products of the bacteria, was first tested for the presence of the enzyme. The enzyme was then precipitated by adding to the filtrate an equal volume of a 5 per cent aqueous solution of safranine. This was filtered through a Buchner filter, washed several times with absolute alcohol, and dried. The protease is thus quantitatively removed from the broth, as is shown by the fact that the filtrate is devoid of all proteolytic powers. This precipitate consists of the protease combined with the safranine and also a small fraction of the proteins which are also precipitated by the azine bases. Amino acids, pepteds, or peptones do not react with the azines. They, therefore, must be related to the more highly organized proteins. A further study of this protein is being made for the reason that having the same reaction with the azine bases, it is probable that it may have other chemical and physical properties in common with the proteases.

The precipitate from the broth culture of *B. proteus* is small, being only a few milligrams per 100 cc. of medium. It is bluish in color and insoluble in any solvents thus far used. Marston dissolved the safranine precipitate of trypsin and pepsin in a M/15 solution of sodium phosphate pH 7.5, but this solution had no effect on the bacterial protease safranine compound.

The precipitate is, however, soluble in the presence of proteins and it is probable that the compound is also dissociated. The precipitate is very active proteolytically; minute quantities of it will rapidly liquefy relatively large amounts of gelatin. The enzyme in combination with the azine base seems to be quite stable, retaining its proteolytic powers for at least one month, the longest interval at which it was tested.

Other azine bases will be used as precipitants, and of the many such bases it would seem at this time that three of them would be especially interesting to try. These three are paradiazine, phenazine and diketopiperazine. Paradiazine is one of the simplest of the azines, and if it acts as a precipitant of the protease, it is probable that the resulting compound would be more soluble, and more easily dissociated than the compounds formed by the more complex azine bases. This would be a distinct advantage in the attempt to purify the enzyme.

Phenazine has a structure similar to that of neutral red in that it has the azine nucleus with two nitrogens in the paraposition and two benzine rings attached but differs from it, and all other azine bases thus far used, in that it has no amino groups attached. Therefore if phenazine precipitates the protease it will be evidence in favor of the theory that the azine nitrogen is the point of attachment. If it does not precipitate the protease this would indicate that the nitrogen was not, and that possibly the amino group was the point of union.

Abderholden[4] suggested that proteins were not straight chains, but rather were composed of ring compounds being anhydrides of amino acids, or that proteins were essentially polydiketopiperazines in structure. Marston[3] also enunciated this theory and by it explained the chemistry of proteolytic enzyme action. In the light of this theory it will be well worth while to study the reactions of diketopiperazine and proteases.

This is a preliminary report.

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[1] Robertson, *J. Biol. Chem.*, 1907, ii, 343.

[2] Holzberg, *J. Biol. Chem.*, 1913, xiv, 335.

[3] Marston, *Biochem. J.*, 1923, xvii, 851.

[4] Abderholden, *Z. physiol. Chem.*, 1923, cxxviii, 119.

3597

Blood Sugar Changes in Avian Polyneuritis.

H. E. REDENBAUGH. (Introduced by A. C. Ivy.)

From the Department of Physiological Chemistry, Northwestern University Medical School.

One of the most striking symptoms found at autopsy of a polyneuritic pigeon, is the remarkable condition of the alimentary canal. The entire gastro-intestinal tract is gorged with food, yet the pigeons appear to be starving. The small intestine in the region of the pancreas atrophys to such an extent that the wall is almost transparent. The pancreas also atrophys. The typical coral pink color of health changes to a dead white or gray and small hemorrhages are generally visible. When examined histologically, the pancreas shows marked changes. McCanison[1] regards pancreatic insufficiency as one of the most important of the effects of food deficiency.

There is also in polyneuritic pigeons a marked decrease in efficiency of the digestive enzymes. This investigation was undertaken in order to determine what effect, if any, the above changes would have on blood sugar. Funk and von Shonborn[2] reported hyperglycemia in pigeons when placed on a diet deficient in vitamin B for 14 days. Funk[3] later repeated the work in an attempt to determine what effect extracts of the ductless glands would have on polyneuritis. He says, "each of the substances to be tested was administered to pigeons on normal food (serving as controls), and to pigeons fed on polished rice for several days, after which the animals were bled from the heart by means of a canulae under A. C. E. anesthesia." The effect of ether anesthesia on blood sugar is well known. The pigeons in the last investigation were fed polished rice but twelve days. In neither group was the stage of convulsive attack reached.

Eggleton and Gross, studying the effects of polyneuritis on rats, contradicted Funk's findings. They furnish a graph showing a lowering of the blood sugar after 50 days on Vitamin B deficient diet. While the average of their tabulated results show an increase blood sugar from 0.09 gm. in normal rats to 0.115 gm. in polyneuritic rats after forty days on deficient diet.

In this investigation, blood was drawn from the heart (by means of needle and syringe without anesthetic). All the polyneuritic pigeons were in the acute or convulsive stage when blood was drawn. The blood filtrate was prepared and sugar determined according to the method of Folin and Wu.[5] The diet of the normal pigeon consisted of a commercial chicken feed mixture, while the polyneuritic pigeons received autoclaved grain as long as they would eat it voluntarily. It was then necessary to use forced feeding, 20 grams of the synthetic lard diet of Suguira and Benedict[6] being given each day.

The results of the blood sugar determinations are given in the following table:

TABLE I. Blood Sugar of Pigeons.

	Milligrams of Glucose	Means
Normal	186.9, 187.7, 186.0, 176.2, 198.0, 215.0, 206.1.	193.7
Polyneuritic	241.0, 263.1, 421.0, 422.0, 316.0, 412.3, 555.0, 482.0, 312.5, 296.2, 336.1.	368.7

As is brought out in the table the polyneuritic pigeons show an unmistakable hyperglycemia. The average blood sugar of the polyneuritic pigeon being almost double that of the normal.

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[1] McCanison, "Studies in Deficiency Diseases." 1921. London.

[2] Funk, C., and von Shonborn, V., *J. Physiol.*, 1914, xlviii, 328.

[3] Funk, C., *J. Physiol.*, 1919, liii, 247.

[4] Eggleton, P., and Gross, L., *Biochem. J.*, 1925, xix, 633.

[5] Folin, O., and Wu, *J. Biol. Chem*, 1920, xxxviii, 81.

[6] Suguira, K., and Benedict, S. R., *J. Biol. Chem.*, 1923, lv, 33.

3598

An Undescribed Bile-Expelling Mechanism in the Guinea Pig.

G. E. BURGET AND R. J. BROCKLEHURST.

From the Department of Physiology of the University of Chicago.

The anatomy of the extra-hepatic biliary system in the guinea pig is in general similar to that in other laboratory mammals, but that it possesses certain peculiar features. The system consists of hepatic ducts, gall-bladder, cystic duct and common bile duct; but the latter terminates in a dilatation, or ampulla, which is closely applied to the surface of the duodenum on its postero-superior aspect and is situated about 7 to 10 mm. beyond the pylorus. From near the distal end of this ampulla there arises a small duct which passes directly through the duodenal wall and terminates at the head of a papilla.

We have observed the functioning of these structures immediately after killing and in animals under barbital anesthesia. On turning up the liver we have always found the system distended with bile in both fed and fasting animals; in this condition its walls are translucent. During the observations the parts were kept moist with warm normal saline. With each peristaltic wave in the duodenum a constriction is initiated in the common duct just above its junction with the ampulla, obstructing the duct lumen, and this is followed by a contraction of the ampulla which completely empties it of its contents. After the wave has passed, both sphincter and ampulla relax; the ampulla then gradually refills with bile in about 20 seconds to 1 minute, the rate depending on the existing tension in the gall-bladder and hepatic ducts. This cycle of events is repeated with the next peristaltic wave, the sphincter and the ampulla always contracting powerfully. Movements of the duodenum other than regular peristalsis were never accompanied by contraction of either sphincter or ampulla.

Faradic stimulation of the first part of the duodenum elicited a localized contraction without causing contraction of either sphincter or ampulla. Direct stimulation of the ampulla, if effective, always caused contraction of the sphincter also, whereas weak stimulation of the sphincter might be effective without inducing contraction of the ampulla. Repeated stimulation of the ampulla, alowing it to refill in the intervals, resulted in gradual emptying of the gall-bladder when the hepatic ducts were obstructed. We have never seen spontaneous contractions of the gall-bladder although slight changes in shape have been observed. Stimulation of the gall-bladder itself always gave rise to a localized contraction of its wall. We have also observed that a sudden increase of tension in the ampulla, produced by pressure on the gall-bladder, never gave rise to contraction of the sphincter or the ampulla.

Serial sections have shown that there is little, if any, plain muscle in the wall of the common duct itself until near its junction with the ampulla. Here a thick band of circularly disposed muscle fibers is seen, corresponding exactly in position to the sphincter mentioned above. This muscle is prolonged downwards over the ampulla and clothes it with a well marked circular coat, which, however, is not as thick as that of the sphincter. There is also a thin outer longitudinal muscle coat over the sphincter and ampulla, which is apparently continuous with the corresponding layer of the duodenal wall and passes directly, together with the serosa, from one to the other. Where the ampulla and the duodenum are in contact their circular muscle layers meet as a solid band without any apparent separation. Near the distal end of the ampulla a small funnel shaped opening leads into a short *direct* duct, terminating at the head of the bile papilla (which is formed chiefly by a thickening of the duodenal mucosa). The musculature of the dividing wall between ampulla and duodenum thins out as it approaches the papilla and eventually disappears in the substance of the latter; although the fibers in this region are arranged sphincter-wise, they are very sparse and do not give the impression of a powerful sphincter.

It thus appears that the guinea pig possesses a specialized form of bile-expelling mechanism, in which an ampulla by active contraction discharges its contents directly into the duodenum with the occurrence of each peristaltic wave; regurgitation into the common duct is prevented by the simultaneous contraction of a powerful sphincter. These observations are of interest in view of the fact that, while the gall bladder possesses some degree of contractility, the flow of bile is not at all dependent upon its powers of contraction. The anatomical arrangement described would appear to be a variation of the usual oblique course of the intra-mural portion of the common duct in most other mammals. It is possible that the ampulla, though lying practically outside the duodenal musculature, is in reality homologous with the true Ampulla of Vater.

This is a preliminary report.

3599

The Effect of Emotion on the Basal Metabolism.

HARRY L. SEGAL, HERBERT F. BINSWANGER AND SOLOMON STROUSE.

From the Michael Reese Hospital, Chicago.

The experiments here reported were undertaken as part of a study on the relation of the nervous symptoms and the basal metabolism in exophthalmic goiter. There is evidence that these two may not be manifestatons of the same disturbed function.[1]

Twelve hospital patients in the surgical ward for various disturbances, and with varying degrees of emotional reaction were told that they would be operated on the following morning. No drugs were given. The basal metabolism had been studied and was also done the day before operation. On the morning of operation, metabolism was again taken before the patient left for the operating room. In none of these cases was there any change in the basal metabolism, although a few showed variations in pulse and blood pressure. A second group of twelve patients with the symptoms of toxic thyroid was chosen. Basal metabolisms were done frequently during the course of preparation for operation, which preparation in this group always included the administration of Lugol's solution. A metabolism was again done on the morning before operation and on the day of operation after the patient had been told the time of operation. Again in this series no significant rise of metabolism was seen in any case, nor were there significant changes in pulse or bloodpressure.

It was realized that patients who had been thoroughly prepared for operation by iodine showed no change. Then it was decided to select a small series of carefully chosen toxic goiters who had not been so treated. The results in this group of patients were in striking contrast to those so far reported. Five individuals are in this group. In four cases the rise in metabolism was from 20 per cent to 35 per cent over the metabolism of the day before, and in one case the rise was 132 per cent. This latter patient had a reaction immediately after the metabolism resembling an acute thyroid intoxication, with a marked increase in metabolism two hours after the first test. It dropped to the original level of plus 63 per cent on the following morning. In two other individuals the metabolism also dropped within 24 hours to the original level.

Although these individuals had been told they would be operated on, their nervous reaction varied greatly, some being glad they were to be operated on, others being excited, but the marked rise in metabolism was found in all. In four of these individuals we were able to repeat the experiment after iodine therapy, and in none of the four was there any rise in the basal metabolic rate.

Conclusion: These experiments show very definitely that the average surgical patient and the iodized toxic goiter show no preoperative rise in the basal metabolism and that individuals with toxic goiter not treated with iodine show the possibility of a dangerous rise The significance of this is being further investigated.

This is a preliminary report.

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[1] Strouse, S., and Binswanger, H. F., *J. Am. Med. Assn.*, 1927, lxxxviii, 161-164.

3600

A Qualitative Indicator for the Testis Hormone.*

CARL R. MOORE. (Introduced by F. R. Lillie.)
From the Hull Laboratory of Zoology, University of Chicago.

Lack of progress in the study of the organs of internal secretion has often been due to the fact that sufficiently dependable, easily read indicators for the products of their activity, were not available. In the mammals this is especially true of the gonads, for these can be removed without causing death of the animals or exceptionally striking changes. In time, of course, certain changes occur that are of importance; in the male there is a diminution in size of the prostate, penis, and seminal vesicles when present, and in the female the reproductive tract undergoes retrogression. The psyche of copulation is often independable, for with the guinea pig I have noted males vigorously inclined to the female despite the fact they were castrated months before, and at an age of thirty days. It has likewise been reported that eunuchs, castrated at an early age, retain for years a persistent inclination to, and a degree of satisfaction in, the association with the female.

The work of Stockard and Papanicolaou[1] on the guinea pig, Long and Evans,[2] Allen,[3] and others on the mouse and rat has developed a dependable method for indicating the female hormone by using the vaginal smear as an indicator. The activity cycle of the female rat shown by the work of Wang, Wang, Richter, and Guttmacher[4] likewise gives promise as a dependable test for the female hormone. On the male side, however, the only promising qualitative test that can be relatively quickly applied to mammals is that of the "Activity" record developed along lines as indicated[4] which has been recently applied by Hoskins.[5] How safe an indicator this will finally prove to be is yet to be demonstrated.

During the last three years I have kept records of the motility of spermatozoa in the rat and guinea pig under many varying conditions, as these have developed in my experimental work on the testicle of mammals.[6] In this place I wish to call attention to one phase of the results obtained for the bearing it has upon the problem of hormone indicators.

In the guinea pig the spermatozoa present in the epididymis of

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* This investigation has been aided by a grant from the Committee of Research in Sex Problems of the National Research Council; grant administered by F. R. Lillie.

one side, at the time the testis is removed from it, retain their power of motility if properly stimulated, for a period of two months or longer, provided the animal's opposite testis is present. Since the source of spermatozoa on the operated side (the testis) has been removed it should be clear that each spermatozoon present was in the epididymis at the time of testis removal, and has therefore persisted and retained its capacity for motility for a period longer than 60 days. Under similar conditions, with the exception that both testes have been removed from the animal, the spermatozoa retain their capacity for movement for approximately but half the time (usually for less than 30 days). Since all conditions in the two cases are alike except that *in one case the testis secretion is being supplied to the animal (by one testis) and in the other case not,* it should be evident that *the length of retention of spermatozoon motility* supplies us with an indicator for the presence of the testis hormone.

With this indicator as a tool I have been studying many phases of the problem as they would be at once indicated. Up to the present time, I have records of sperm motility on considerably over two hundred males that involves the many phases of this study. A more extensive report of these studies will appear elsewhere.

Benoit[7] noted that sperm motility persisted longer in the isolated epididymis when the testes were present than when they had been removed; this paper was not available to me until the last few months. Though noting the same effects that I have described, he makes no mention of its application for further work. It appears, from his findings, that certain cytological pictures of cells of the epididymis may afford a means of reading the results of experimental procedures even quicker than the sperm motility reaction. Since reading his paper, I have been making an attempt to extend the indicator along these lines.

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[1] Stockard, C. R., and Papanicolaou, G. N., *Am. J. Anat.*, 1917, xxii, 225-264; *Biol. Bull.*, 1919, xxxviii, 222-245.

[2] Long, J. A., and Evans, H. E., *Mem. Univ. of Calif.*, 1922, vi,

[3] Allen, Edgar, and others, *Am. J. Anat.*, 1924, xxiv, 133-182.

[4] Wang, G. H., *Comp. Psychol. Monographs*, 1923, ii, Ser. No. 6, 1-26. Wang, G. H., Richter, C. P., and Guttmacher, A. F., *Am. J. Physiol.*, 1925, lxxiii, 581-599.

[5] Hoskins, R. G., *Am. J. Physiol.*, 1925, lxxii, 324-330; *Endocrinology*, 1925, ix, 277-295.

[6] For a review of much of this work see Moore, *Quat. Rev. in Biology*, 1926, i, and subsequent papers.

[7] Benoit, *Thesis, U. of Strasbourg*, 1925.

3601

Hemolytic Action of Beef Serum in Relation to Age of Cattle.

D. A. KOTSOVSKY. (Introduced by Nathaniel Kleitman.)

From the Department of Physiology of the University of Chicago.

In this study the hemolytic action of old cattle serum with that of calf serum was compared. Beef blood was obtained at the Stock Yards. It was drawn directly from the large neck blood vessels and immediately defibrinated. The blood was then centrifuged, and the serum tested for hemolytic power not later than 6 hours after the death of the animal. Rabbit red blood cells were washed repeatedly with physiological salt solution, and one cubic centimeter of a 5 per

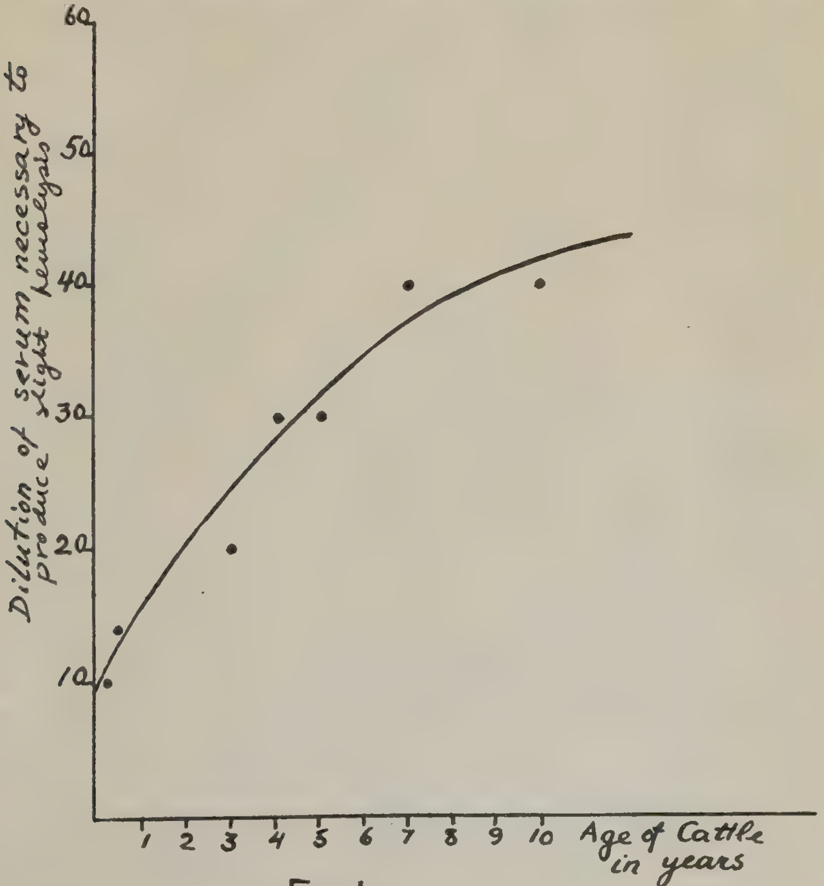


Fig. 1

cent suspension of these cells was mixed with one cubic centimeter of diluted beef serum (1:10). If the hemolysis was complete, the serum was further diluted until it no longer caused any hemolysis of the rabbit erythrocytes. The serum of 6 adult cattle of various ages, two calves, and three embryos was tested. Fig. 1 shows the increasing hemolytic power of the serum of cattle with increasing age. Fig. 2 shows the comparison between the hemolytic action of serum from 5-months old calf and a 12-year old ox. It will be seen that in a dilution of 1:20 the "old" serum produces complete hemolysis, while "calf" serum in the same concentration has no hemolytic power whatever.

Addition of "young" serum to "old" only partially neutralizes the hemolytic power of the latter. This was found to be the case in four different experiments.

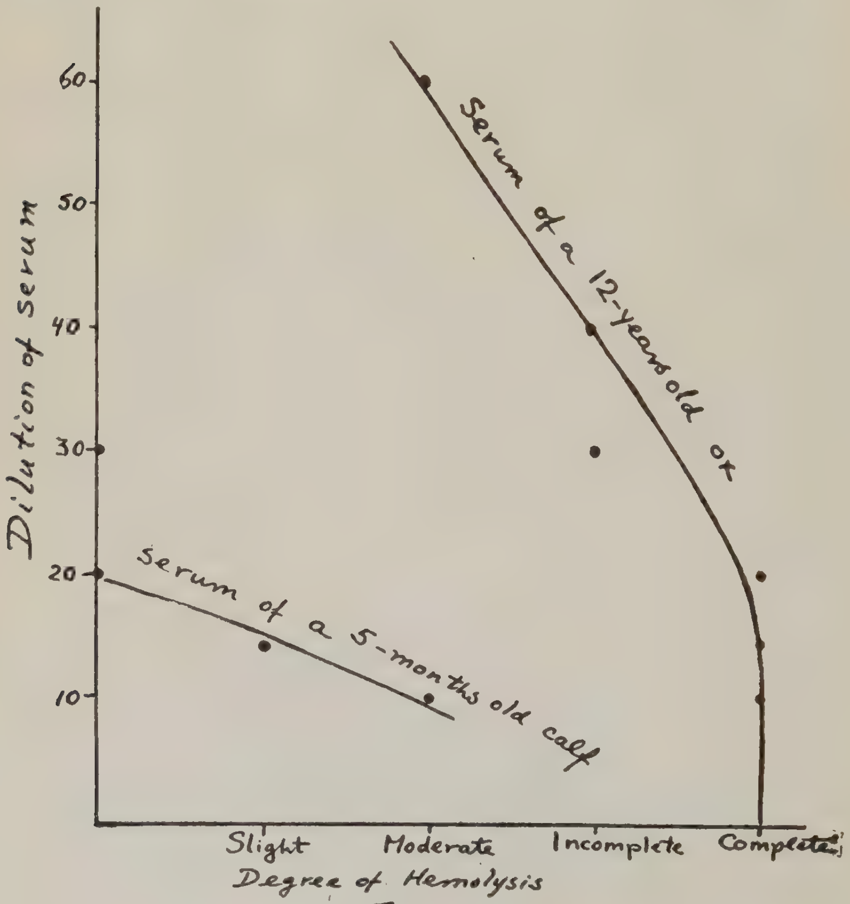


Fig. 2

The hemolytic power of beef serum that is kept in the ice box (3° C.) remains unchanged for 48 hours, then begins to deteriorate. It is thought that the marked difference between the hemolytic power of serum from animals of different ages may be an example of other changes accompanying old age, and further tests are being planned to bring these to light, and thus, perhaps elucidate the cause of senility.

This is a preliminary communication.

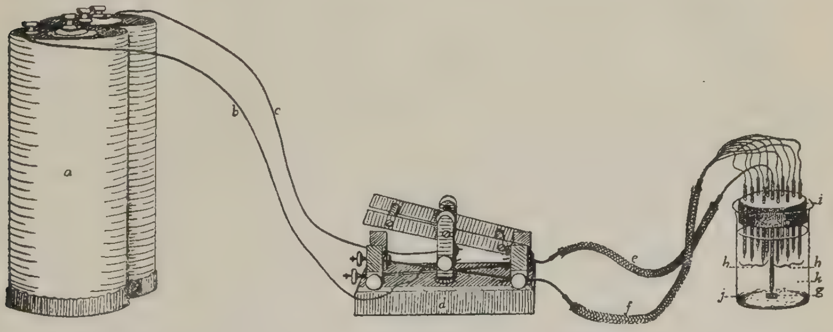
3602

A Simple Arrangement for Platinizing Electrodes.*

WILLIAM C. AUSTIN.

From the Department of Physiological Chemistry, Loyola University School of Medicine.

The accompanying figure shows a simple arrangement for the electrolytic deposition of platinum upon electrodes for hydrogen ion work.



A battery of two dry cells, *a,* is connected in series to a commutator, *d,* by wires, *b,* and *c.* The commutator is connected to a single central anode, *g,* by wire *e,* and to the cathodes, *h,* by branching of the cable wire, *f.* The electrodes, *g,* and *h,* are loosely held in position by insertion through the holes of the flat cork, *i,* which is supported in the 100 cc. beaker, *j,* in such a way that the metal of the electrodes is immersed in the platinic chloride solution, *k.* The electrode, *g,* consists of a platinum foil connected to a short piece of platinum wire, which is in turn sealed into the 5 mm. glass tube. A single wire electrode would probably serve just as well here. The electrodes, *h,* were made by sealing 18 or 20 gauge platinum wire into pieces of 4 mm. Mett's tubing. The cable wire, *f,* is a piece of 16 strand electric wire such as is used in flexible extension cords. The core has been bared and the individual strands distributed as shown.

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* Demonstrated at the Federation of American Societies of Experimental Biology, Rochester, N. Y., April 15, 1927.

The arrangement has several distinct advantages.

1. Many electrodes may be prepared simultaneously at a great saving of time.
2. After plating is concluded the cork with electrodes may be transferred to a 100 cc. beaker filled with distilled water.
3. A reserve stock of electrodes is always available. The electrodes thus prepared keep for several months in distilled water.
4. The apparatus is in stable equilibrium.

In removing the prepared electrodes care must be exercised to draw them downward and out in such a way as to avoid scraping the plated surfaces. The holes in the cork should not grip the electrodes too tightly. Wetting will aid in removing them and inserting them into the rubber stoppers of the Clark vessel.

In all other respects the directions for preparation of the electrodes do not differ from those of the accepted treatises.

From theoretical considerations one would expect that the electrodes nearer the central anode would receive heavier deposits of platinum. In order to avoid this as much as possible, the anode is lowered to the bottom of the solution. In actual practice we have not encountered any difficulties due to inequalities of deposits of platinum.

Western New York Branch.

University of Syracuse Medical School, May 14, 1927.

3603

An Adaptation of the Electron Tube to the String Galvanometer.

C. J. CAMPBELL. (Introduced by F. P. Knowlton.)

From the Physiological Laboratory, College of Medicine, Syracuse University.

In coupling the string galvanometer to the output of an electron tube, various devices have been used to avoid placing the string directly in the output circuit.[1, 2] This has been done to avoid damage to the string because of the high plate voltages used. However, the voltage amplification factor (μ) which is one of the most important constants in amplification equations, is a function of the geometry of the tube. $\mu = \dfrac{2\pi p}{a \log_e \frac{a}{2\pi r}}$ approximately[3] where $p =$ distance from grid to plate, $a =$ distance between adjacent wires of grid, $r =$ radius of grid wires. Practically μ is found to vary but little within a wide range of plate voltage. Therefore, there is only slight loss of amplification involved in operating at low plate potentials, and there is considerable gain in safety of manipulation. Furthermore, in operating at low plate potentials and normal filament temperatures, one is working on that part of the characteristic of a tube where the plate to filament current is limited by space charge. This tends to reduce fluctuations in the tube circuit which are due to the so called "shot effect."[4]

When low plate potentials are used, the galvanometer may be placed directly in the output, and zero position of the string maintained by applying to the string terminals a potential equal and opposite to the P. D. set up by the passage of the plate current.

The circuit adopted is shown in Fig. 1. It consists of one stage of voltage amplification and one stage of power amplification. The resistances used are practically pure resistance and when the string is placed directly in the output circuit the amplifier is aperiodic. Con-

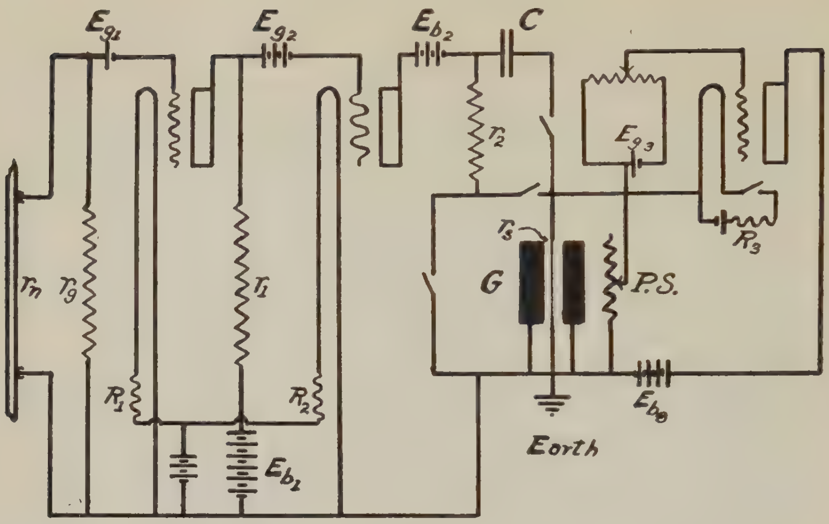

FIG. 1.

Conventional symbols throughout. r_n tissue resistance. r_g a non inductive resistance of from $10^4\omega$ to $2\times10^6\omega$ depending on stability of circuit and amplification desired. $r_1 =$ plate to filament resistance of first tube × 5. $r_2 + r_g =$ plate to filament resistance of second tube. R_1, R_2, and R_3 are ballast resistances. C is a 2 mfd. condenser. P. S. a protective shunt. μ of first tube = 27. μ of second tube = 3.1. E_{b1} 90 volts. E_{b2} 22.5 volts.

stant current amplification of from slightly less than unity to some two hundred times may be secured, depending on the tissue resistance and the value of r_g used. Compensation is effected by means of a third vacuum tube which acts as a variable high resistance.[5]

When recording slowly changing potential differences, a heavy string of 800ω resistance direct coupled to the amplifier is used. When rapid changes of potential lasting 10 σ and less are recorded, a light, high resistance string is used coupled to the amplifier through a capacity of 2 mfd. This does away with the necessity for compensation of the plate current, and at the frequencies mentioned no appreciable distortion is introduced.

This is a preliminary report.

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[1] Forbes and Thatcher, *Am. J. Physiol.*, 1920, lii, 409.

[2] Chaffee, Bovie and Hampton, *J. Optical Soc. Am.*, 1923, vii, 1.

[3] Van der Bijl, "The Thermionic Vacuum Tube," New York, 1920, pp. 229 *et seq.*

[4] Hull and Williams, *Phys. Rev.*, Feb., 1925, pp. 166 *et seq.*

[5] Steinhausen, *Pflüger's Archiv.*, 1920, clxxxv, 703.

3604

Lipoids in the Kidney of Adrenal Insufficiency.

C. G. MAC ARTHUR, G. A. DEAN AND F. A. HARTMAN.

From the Laboratories of Physiology and Pharmacology of the University of Buffalo.

The presence of large amounts of lipoid in the cortex of the kidneys of adrenalectomized cats has been described.[1] The staining reactions indicated the presence of cholesterin-esters, cholesterin-fatty acid mixtures, and neutral fats.

A study has been made of the chemical nature of these substances. Thirteen completely adrenalectomized cats and 8 normal cats were used in this work. The adrenalectomized cats survived an average of 12.7 days, the survival periods varying from 5 to 26 days. Some of the animals were injected daily with adrenal extracts.

For the chemical analysis, one gram of fresh, ground kidney is extracted with a solution of chloroform and alcohol, 1-3, and made up to 20 cc. Aliquot parts are taken for the following determinations. One portion is evaporated to dryness and weighed for *total lipoids*. The *free acid* is titrated directly with .01 N NaOH. In calculating the weight, 280 is taken as the molecular weight. The volatile acids are separated from the non-volatile by saponifying, acidifying, and aerating over a boiling water bath. The residue is titrated with .01 N NaOH for non-volatile acids and the contents of the receiving tube for the volatile acids. The molecular weight of the non-volatile constituents is taken to be 280, that of the volatile to be 100. Cholesterol and phosphorus are determined by the standard colorimetric methods. The iodine value is reported in milligrams of iodine absorbed per gram of original kidney material.

TABLE I. *Results, per gram of kidney.*

	Average in normal cats	Average in adrenalectomized cats	Difference
	mg.	mg.	Per cent
Total lipoids	70.5	107.1	51.9 increase
Free acids	9.97	20.97	110.4 ”
Volatile acids	1.86	5.29	184.3 ”
Non-volatile acids	40.15	50.49	25.6 ”
Cholesterol	2.16	3.83	77.7 ”
Iodine (absorbed)	26.14	30.50	16.7 ”
Phosphorus	1.40	1.01	27.8 decrease

The per cent increase in iodine absorbed in the kidneys of adrenalectomized cats is less than the per cent increase in the total lipoids. Therefore, the extra fat contains a smaller proportion of double bonds than the normal. The kidney phosphorus decreases after removal of the adrenals, even though the total lipoids markedly increase. This may mean that there is a cellular degeneration along with the deposition of less active fat. If one calculates the amount of fatty acids derived from the phospholipoids and compares it with the non-volatile acids, in the normal about two-thirds of the fatty acids are from the phospholipoids and in the operated animals only about one-third.

The chief source of increase in the ordinary fatty acids is in the free fatty acid fraction. The neutral fats have increased too, but to a lesser extent.

The most striking fat change is in the volatile fraction. The amount of these esters of the lower fatty acids is small in normal kidneys, but is about three times as large in the operated animals.

Though the total cholesterol in the adrenalectomized animals is 78 per cent larger than in the normals, the increase in milligrams is small. Whether this increase is an ester or free cholesterol is yet to be determined.

A similar chemical study is being made of the lipoids in blood and urine.

This is a preliminary report.

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[1] **Hartman, F. A., MacArthur, C. G., Gunn, F. D., Hartman, W. E., and MacDonald, J. J.,** ***Am. J. Physiol.*****, 1927, lxxxi, 244.**

3605

Simultaneous Recording of Peristalsis and Action of Pyloric Valve in Isolated Stomach of Frog.

JESSE SERBY AND M. S. DOOLEY.

From the Department of Pharmacology, Syracuse University.

The method consists essentially of enclosing the excised stomach in a small volume recorder (a 2-way dog size arterial cannula serves the purpose). A sufficient length of oesophogus and intestine are removed with the stomach to permit introduction of an inflow cannula into the former and an outflow cannula into the latter in a way not to interfere with the valves at either end of the stomach. The stomach is then passed into the "Oncometer," short segments of rubber tubing serving to make an air tight seal between the cannulae and the "Oncometer." From the side tube of the latter a rubber tube leads to a delicate tambour which records both peristaltic waves and tone changes. Perfusion of the lumen of the stomach is effected from a constant pressure tank in which the nature of the fluid can be easily altered. The optimum height of the tank is about 15 cm., the flow ceasing at a height of 8 to 9 cm. A flutter valve is placed between the pressure tank and the stomach to prevent back flow, when peristaltic waves press against a closed pylorus or during antiperistalsis. The operation of the pyloric valve is recorded by placing a drop-counter beneath the tip of the outflow cannula.

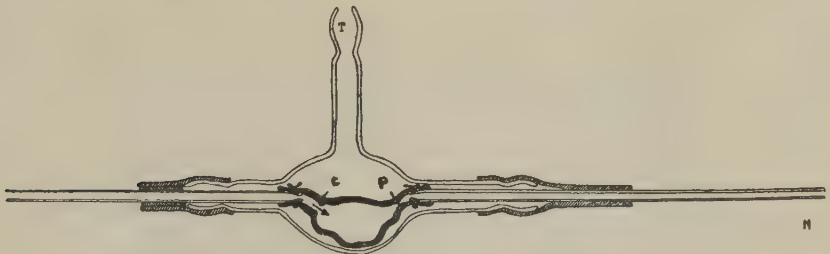

Fig.I-Shows method of enclosing stomach in air-tight chamber. C=Cardia;, P=pyloric valve. T=connection to tambour.

The method has the advantages of freedom from indwelling balloons or other similar interferences, and permits direct observations, especially by the aid of a reading glass, at all times.

The feasibility of such an arrangement for the demonstration of normal functions of the stomach and also of the influence of drugs is apparent. A report on such is in preparation.

3606

Increase of Blood Platelets in Rabbits by a Specific Bacterial Infection.

FRANCIS D. GUNN.*

From the Laboratory of the Buffalo General Hospital.

In the course of an experiment on rabbits in which the effect of splenectomy upon blood platelets was being studied, a peculiar caseous necrosis about the site of operation was encountered in one

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* Fellow in Medicine, National Research Council.

TABLE I.

Rabbit	Mode of inoculation	Result	Pathological changes	Platelets		Percentage of spindle forms		Leucocytes	
				Average normal	Maximum	Average normal	Maximum	Average normal	Maximum
No. 22 brown 1641 g.	Intraven. and subcut.	Died spontaneously	Caseous pneumonia; caseous necrosis at site of subcutaneous injection.	980,000	1,757,000	3	13		8,450
No. 23 albino 2179 g.	Intraperit. subcut. intramusc.	Recovered		1,185,000	2,400,000	5	13	9,200	15,000
No. 24 albino	Subcut. and intramusc.	Died spontaneously	Caseous pneumonia; extensive caseous necrosis at site of injection	662,000	1,739,000	4	12		
No. 25 albino 2547 g.	Subcut. and intramusc.	Recovered		1,171,000	1,459,000	4	10.2	8,760	10,450
No. 26 black and white 2377 g.	Subcut. and intramusc.	Died spontaneously	Caseous pneumonia; caseous necrosis at site of injection; accidental infection of pleura and pericardium (pneumococcus)	1,112,000	1,680,000	8.5	15.5	4,800	35,000
No. 27 black 2352 g.	Subcut. and intramusc.	Recovering; autopsied on 15th day	Extensive caseous necrosis at site of injection	580,000	1,965,000	2.5	12	8,500	23,000

of the animals. This was found to be due to an infection with a motile, Gram-negative bacillus. From the studies made on the bacillus so far, it can with certainty be excluded from the Salmonella group; moreover it cannot be identified with any of the human pathogenes. Further studies are in progress and will be reported upon later. Because of an unusual increase in number of blood platelets in the infected animal, six healthy rabbits were inoculated with the cultured micro-organisms and the effect upon platelets observed.

Results. The accompanying Table I shows the change in number of platelets and leucocytes in these six animals and indicates the pathological changes in the four which were examined post mortem. The greatest increase in platelets in any one animal was 3.4 times its normal, while the greatest so far obtained with ultra-violet light is 2.3 times. The greatest increase in three splenectomized rabbits was 2.5 times the normal.

The maximum leucocyte number found in the uncomplicated cases was 23,000, which was observed in Rabbit 27. Of this number about 65 per cent were small lymphocytes, whereas in Rabbit 26, which developed an accidental pyogenic infection, over 70 per cent of the 35,000 leucocytes were polymorphonuclears.

It was noted in a previous communication on the influence of ultra-violet light upon the blood platelets[1] that the small, spindle-shaped platelets were greatly increased during the so-called platelet crisis, *i. e.*, at the height of the platelet increase. Differential counts have been made in these experiments also, and the spindle forms consistently showed a relative increase at the time of the increased platelet number, especially at the height of the increase. The number in the normal is usually below 5 per cent of the total, and rises during the platelet crisis to 10 to 15 per cent. If the assumption is correct that these slender, spindle-shaped platelets represent young or immature forms, it may be concluded that the increase is due to increased production rather than to diminished destruction, as is assumed to be the case in splenectomy[2] and as might be assumed in an infection on the basis of a blocking of the reticulo-endothelial cells by the invading bacteria. The erythrocytes showed no constant alteration in number in these experiments.

This is a preliminary report.

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[1] Gunn, F. D., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 120-123.

[2] Bedson, S. P., *Lancet*, 1924, ii, 1117.

3607

Simple Experimental Anemia and Liver Extracts.

G. H. WHIPPLE AND F. S. ROBSCHEIT-ROBBINS.

From the Department of Pathology, the University of Rochester School of Medicine and Dentistry.

In 1920 Whipple, Robscheit and Hooper[1] reported that liver feeding had a very favorable influence on the production of red cells and hemoglobin in dogs during short anemia periods. Since that time Whipple and Robscheit-Robbins have shown that in long standing severe anemia in dogs a liver diet has an extraordinary effect. In fact a diet of liver (beef, pig, sheep, calf or chicken) will produce a maximum regeneration of blood hemoglobin and red cells under standard experimental conditions.[2]

During this considerable experimental period we have tested from time to time a variety of liver extractives. Dr. Hooper in San Francisco tested an alcohol-ether extract upon a few cases of severe pernicious anemia with results which at that time were believed to be negative. In our work we have paid particular attention to changes in the hemoglobin index during these diet periods. We have used a considerable variety of food materials and extracts in an effort to modify the hemoglobin index. The hemoglobin index is obtained by dividing the hemoglobin expressed in per cent by the red cell hematocrit expressed in per cent. At times it appeared that we could decrease the hemoglobin index by certain foods or extracts (Table I) which might indicate a more rapid formation of stroma than of hemoglobin so that the cells would be less completely saturated with hemoglobin. Again we noted an increase in the hemoglobin index which might indicate a more rapid production of hemoglobin than of stroma. These changes are not great and are inconstant, but we believe these changes are significant and in time will be fully understood.

Changes in the hemoglobin index are of particular interest in relation to the recent important work of Minot and Murphy,[3] who give evidence that liver diet is important in the clinical treatment of pernicious anemia. From the standpoint of the pathologist we have pointed out[4] the observed facts in pernicious anemia which in our judgment indicate a *faulty blood construction* rather than an *increased blood destruction* as the essential feature in this obscure disease. The experiments of Cohn, Minot, Fulton, Ulrichs, Sargent, Weare and Murphy[5] tend to support this view and the evidence may finally show that in pernicious anemia we are dealing with a real *deficiency disease* in which the stroma construction material is wanting.

Because of the work of Minot and his colleagues dealing with the influence of certain liver extractives upon human cases of clinical pernicious anemia, we felt it would be of interest to report briefly some of our work on liver extracts as applied to simple, long-continued secondary anemia in dogs. It will be seen from the tables below that all the liver fractions contain some potent substances which are capable of influencing somewhat the regeneration of hemoglobin and red cells under standard conditions. The sum of these reactions does not equal the favorable reaction of whole liver feeding, so that one may assume considerable loss in the method of extract preparation.

Method.

The general experimental method has been described elsewhere.[6] The liver fractions were prepared as follows: To 1000 grams of fresh ground beef liver were added 3000 cc. of 1/10 per cent HCl—the mixture brought to a boil and continued boiling for 2 minutes. The clear filtrate was concentrated to about 1/4 the original volume

TABLE I.
Water extract of beef liver.

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REM'V'D BLED
Food, grams per day	*%*	*kgm.*	*cc.*	*mil.*			*%*	*%*	*gm.*
Dog 24-26 Bull, male, adult.									
Fresh apples 200, bread 300	88	10.4	592	4,6	0.42	2.01	19.4	39	1.1
Fresh apples 200, bread 300, salm. 75	85	10.0	606	4,8	0.45	2.05	20.9	43	1.3
Water liver ext. 500 cc. (eq. 500 g. fr. liver), bread 400, salm. 50	100	10.6	594	5,7	0.45	2.06	21.2	44	13.1
	100	10.7	594	5,2	0.48	1.90	21.5	41	11.6
Bread 400, salm. 50	98	10.6	580	5,4	0.43	1.99	23.3	46	1.3
Dog 24-49 Bull, female, adult.									
Bread 400, salm. 75	100	14.2	786	5,8	0.37	1.83	23.7	43	1.3
Bread 400, salm. 75	96	14.7	828	5,5	0.45	2.11	19.9	42	15.3
Water liver ext. 500 cc., bread 400	100	14.5	806	5,5	0.43	2.01	23.5	47	1.4
Water liver ext. 500 cc., bread 400	100	14.2	808	6,6	0.42	1.81	22.1	40	20.4
Bread 400	100	14.2	808	5,8	0.39	1.89	24.3	46	1.3

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}$$

over steam. This represents the water extract given in Table I—fed readily by mixing with the dry standard bread.

The coagulated liver brei remaining was then extracted with 2 volumes of 95 per cent grain alcohol at 38° C. for 24 hours—a second extraction was done in similar fashion and these two alcoholic extracts combined and evaporated over steam to a thick paste. This fraction represents the alcoholic extract used in Table II.

The residue of liver after water and alcohol extraction as described above was freed from alcohol by boiling with water. This is the liver residue fed in Table III.

Table I shows that this watery extract of liver contains some materials favorable for hemoglobin and red cell regeneration in standard anemia conditions. Dog 24-26 ate all of the food mixture and shows an increased production per 2 week period of about 20 grams of hemoglobin above the base line level of control periods. Dog 24-49 shows about the same increased production of 20 grams above control periods. The increase of 15 grams hemoglobin in the control fore-period is due to a "carry over" from the preceding favorable diet period not shown in the table (diet of dried apricots). We observe a *fall in the hemoglobin index* in both experiments which may indicate that stroma production may be in advance of hemoglobin production. These dogs received a watery extract, the equivalent of 500 grams fresh liver daily, added to a ration of standard bread 400 grams daily. Dog 24-26 received in addition 50 grams canned salmon daily as an appetizer.

TABLE II.
Alcoholic extract of beef liver.

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REM'V'D BLED
Food, grams per day	*%*	*kgm.*	*cc.*	*mil.*			*%*	*%*	*gm.*
Dog 24-22 Coach, female, adult.									
Bread 375, salm. 50	100	11.9	700	5,5	0.40	1.94	22.7	44	1.3
Alcoh. liver ext. 26, br. 400	100	12.0	648	5,5	0.43	1.85	25.4	47	1.3
(eq. to 500 g. fresh liver)	100	12.0	664	6,0	0.42	1.86	29.4	55	14.2
Bread 400, salm. 50	97	12.0	653	5,2	0.48	1.86	22.9	43	12.0
Bread 400, salm. 50	96	12.1	730	4,7	0.49	2.03	22.7	46	1.2
Dog 18-114 Bull, female, adult.									
Alcoh. liver ext. 26, br. 350	94	15.0	779	4,4	0.53	2.04	22.8	46	1.5
(eq. 500 g. fr. liver), salm. 75	90	15.2	819	4,9	0.53	2.08	22.0	46	17.7
Bread 375, salm. 75	67	14.5	714	4,8	0.47	2.00	22.5	45	1.4

Table II shows that the alcoholic extract likewise contains some material favorable for hemoglobin and red cell regeneration. Dog 24-22 ate all of the material and shows an increased production per 2 week period of about 22 grams of hemoglobin above the control periods. Dog 18-114 under the same condition shows a little less production of hemoglobin—about 16 grams per 2 week period. This dog did not eat quite all the material. These dogs received an alcoholic extract the equivalent of 500 grams fresh liver daily added to a ration of standard bread.

TABLE III.
Beef liver residue after water and alcoholic extractions.

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REM'V'D BLED
Food, grams per day	%	*kgm.*	*cc.*	*mil.*			%	%	*gm.*
Dog 24-25 Bull, male, adult.									
Bread 400	100	13.0	733	5,7	0.43	1.96	25.2	49	1.5
Liver residue 275, bread 300	87	13.7	815	6,2	0.43	1.86	26.8	50	14.8
(eq. to 500 g. fresh liver)	100	13.8	717	6,4	0.47	1.90	26.8	51	18.7
Bread 400	100	13.8	778	5,0	0.49	2.08	23.5	49	1.3
Bread 400	100	13.6	778	5,7	0.48	1.92	28.6	55	13.1
Dog 19-104 Bull, male, adult.									
Bread 275, salm. 75	84	11.3	634	4,8	0.50	2.11	22.7	48	1.4
Bread 275, salm. 75	84	11.3	652	4,5	0.49	2.06	21.2	44	1.2
Liver residue 275, bread 225	77	11.7	720	4,1	0.52	1.89	22.7	43	1.1
Liver residue 150, bread 200	55	11.2	622	6,3	0.48	1.97	22.1	44	17.1
Bread 250, salm. 75	47	10.3	615	5,2	0.42	1.95	22.5	44	1.3

Table 3 shows that the liver residue after water and alcoholic extraction still contains substances favorable for hemoglobin and red cell regeneration in standard anemia conditions. Dog 24-24 ate practically all of this residue and shows an increased production per 2 week period of about 35 grams of hemoglobin above the control level. This includes a "carry over" of about 12 grams in the second week after-period due to the reserve stored during the favorable diet period of liver residue feeding. Dog 19-104 ate about two-thirds of the liver residue, was given less during the second week, and partly on that account, produced less hemoglobin per 2 week period, about 15 grams.

From these few experiments one may not draw conclusions, but the following points may be made with some security. All three of these fractions prepared from fresh liver contain substances which are favorable for hemoglobin and red cell regeneration in simple anemia in dogs. In round numbers we may say the watery fraction produces per 2 week period in excess of about 20 grams—the alcoholic fraction perhaps a little less than 20 grams of hemoglobin and the liver residue perhaps 20 to 30 grams of hemoglobin per 2 week period above control levels. The sum of the three extracts would be about 60 to 70 grams hemoglobin per 2 week period. This corresponds to 80 to 100 grams hemoglobin per 2 week period which would be about the average value of the fresh liver fed to the same standard anemia dogs.

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[3] Minot and Murphy, *J. Am. Med. Assn.*, 1926, lxxxvii, 470.

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[5] Cohn, Minot, Fulton, Ulrichs, Sargent, Weare and Murphy. Paper read before the Federation of American Societies for Experimental Biology, Rochester, N. Y., April, 1927.

[6] Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Physiol.*, 1925, lxxii, 395.

3608

Effect of Extracts of Pars Tuberalis of Hypophysis on Urine Secretion.

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Saline suspensions or boiled acidulated extracts of the bovine *pars tuberalis* when injected intravenously into rabbits under paraldehyde anesthesia produce a diuresis differing from that produced by injection of posterior lobe extracts. The resulting diuresis comes on without a marked rise of blood pressure and without the latent period of 3 to 5 minutes (during which the flow of urine is entirely suppressed) which are characteristic accompaniments of posterior lobe diuresis.

Diabetes insipidus is held[1, 2] to be due to injury of the base of the brain in the region of the *tuber cinereum*. Critical examination of the figures of Bailey and Bremer,[2] and of Curtis,[3] shows that in practically every instance the injury has involved the *pars tuberalis* of the hypophysis.

Bourquin[4] has shown that experimental *diabetes insipidus* in dogs is an irritation rather than a deficiency phenomenon. Slight cauterization of the floor of the third ventricle produces the diabetes but deeper cautery destroys it. She further concludes that "the diuresis must be due to a substance produced at the site of the causative disturbance" for the *diabetes insipidus* "runs its typical course after trans-section of the spinal cord at the level of the eighth cervical vertebra, double vagotomy below the diaphragm, and paralysis of the parasympathetic nervous system with atropin."

If the cause of experimental *diabetes insipidus* is to be ascribed to the irritative production of a hormone in the floor of the third ventricle, it is no less reasonable to suppose that the hormone is derived from the *pars tuberalis,* which is distinctly a glandular structure, than that it is produced by brain tissue.

The participation of the *pars tuberalis* in the causation of *diabetes insipidus* has not been ruled out by those who injure the floor of the brain between the optic chiasm and the mammillary bodies. In this connection the action of *pars tuberalis* extracts on urine secretion is of interest.

This is a preliminary report.

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3609

Influence of Pituitrin on Diuresis Variously Induced.

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The diuretic action of extracts of the pituitary body was first described by Magnus and Schafer.[1] Their finding has been confirmed repeatedly. Von den Velden[2] showed that pituitrin may exert an opposite or antidiuretic effect. Reasoning that the polyuria of *diabetes insipidus* was caused by pituitary deficiency, he further used pituitary extracts successfully in the control of the polyuria. Recent papers by Smith and McClosky,[3] Fromherz,[4] and Stehle[5] give a review of the literature. As yet there is no general agreement as to the explanation of the apparently contradictory results.

TABLE I. *Influence of Pituitrin on diuresis variously induced.*

Exp. No.	Form of Diuresis and Injection Rate cc. Ringer per min.	Pituitary Extract	Period Before Pituitrin		Injection of Pituitrin		Period After Pituitrin		Remarks.
			Length of Period Min.	Urine Drops per 5 Min.	Length of Period Min.	Urine Drops per 5 Min.	Urine Drops per 5 Min. First hr.	Urine Drops per 5 Min. Sec'd hr.	
1.	1.47	Infundin, 1-100 R.* Iv.	90	89	80	255	130		
2.	1.72	Infundin, 1 cc. Im.	110	35	60	116	25		
3.	1.73	Infundin, 1 cc. Im.	150	102	60	198	79		
4.	1.77	Pituitrin, 1 cc. Im.	120	26	60	133	40		
5.	1.72	Pituitrin, 2 cc. Im.	95	49	60	167	93		
6.	2.19	Pituitrin, 1-50 R. Iv.	185	35	35	261	147	89	
7.	2.54	Pituitrin, 1-400 R. Iv.	90	19	100	170	81	74	
8.	2.53	Pituitrin, 1-1000 R. Iv.	115	88	115	179	99	152	
10.	2.54	Pituitrin, 1-5000 R. Iv.	45	25	60	110	107		
11.	2.55	Pituitrin, 1-10000 R. Iv.	25	46	30	120	59		
12.	2.48	Pituitrin, .25 cc. Iv.	10	32	60	309	207	178	Atropine, gr. 1/90
13.	Urea 5% in 0.52	Pituitrin, 1-200 5% urea Iv.	65	81	40	131	85		
15.	Urea 5% in 0.55	Pituitrin 1-100 5% urea Iv.	70	11	35	142	23	48	
16.	Urea 10% in 0.55	Pituitrin 1-100 10% urea Iv.	45	82	15	254	98	81	
17.	Urea 10% in 0.55	Pituitrin, .43 cc. Im.	55	39	60	114	110		
18.	NaCl 5%, 0.40	Pituitrin, 1-100 5% NaCl Iv.	45	68	75	134	44	45	Constantly falling blood pressure
19.	Theocin 0.5%, 0.40	Pituitrin, 1-100 5% Theocin Iv.	40	121	45	122	10		Constantly falling blood pressure
20.	Ringer. 2.5 cc	Pituitrin, 1-1000 R. Iv.	15	36	15	227	123		

* R means Ringer, Iv intravenously, Im Intramuscularly.

TABLE II. *Influence of Pituitrin. Injections repeated at intervals.*

Exp. No.	Initial Period		First Pituitrin Period		Interval		Second Pituitrin Period		Interval		Third Pituitrin Period		Interval		Fourth Pituitrin Period		Remarks.
	Length of Period Min.	Drops per 5 Min.	Length of Period Min.	Drops per 5 Min.	Length of Period Min.	Drops per 5 Min.	Length of Period Min.	Drops per 5 Min.	Length of Period Min.	Drops per 5 Min.	Length of Period Min.	Drops per 5 Min.	Length of Period Min.	Drops per 5 Min.	Length of Period Min.	Drops per 5 Min.	
1.	20	66	15	88	—	—	15	86	—	—	15	84	—	—	15	120	Ringer Diuresis. Pituitrin 0.5 cc. hypodermically. Rate of urine flow increased for an hour after last injection.
2.	20	20	10	112	20	52	10	80	20	34	10	71	20	57	10	70	Ringer Diuresis. 1-1000 pituitrin intravenously.
3.	20	43	10	226	20	150	10	221	20	127	10	219	20	142	10	230	Ringer Diuresis. 1-1000 pituitrin intravenously.
4.	30	161	30	319	40	167	60	231	—	—	—	—	—	—	—	—	Ringer Diuresis. 1-100 pituitrin intravenously.
5.	60	17	60	218	35	55	20	232	—	—	—	—	—	—	—	—	Ringer Diuresis. 1-100 pituitrin intravenousy.
6.	60	37	60	133	60	37	60	96	—	—	—	—	—	—	—	—	Ringer Diuresis. Blood pressure low. 1 cc. pituitrin intramuscularly.
7.	30	16	35	145	30	57	30	100	30	67	—	—	—	—	—	—	5% Urea Diuresis. 1-100 pituitrin intravenously.
8.	30	18	30	136	30	87	15	130	15	73	15	129	—	—	—	—	Ringer Diuresis. 1-200 pituitrin intravenously

The experiments reported in this paper were limited to a study of the action of pituitrin superimposed on a diuresis variously induced, and to a comparison of the effect of variation in dosage and method of administration. It was hoped that such experiments might throw some light on the question of the mechanisms responsible for the antagonistic actions reported. Rabbits anesthetized with morphine and urethane were used throughout the experiments. Cannulae were inserted in the ureters and urine flow recorded by a drop counter. Blood pressure from the carotid was recorded simultaneously. By means of a Woodyatt injecting pump, solutions warmed to body temperature were injected at a uniform rate. In this way diuresis was induced and the effect of pituitrin upon this existing diuresis was studied. When injection is continued in this way at a rate varying from 2.5 cc. per minute to .5 cc. per minute, depending on the solution employed, the diuresis usually begins gradually and mounts slowly to a plateau like level which often may be maintained for some hours, provided blood pressure remains uniform. In a few instances among the controls, the slow rise continued until the end of the experiment. Such instances were

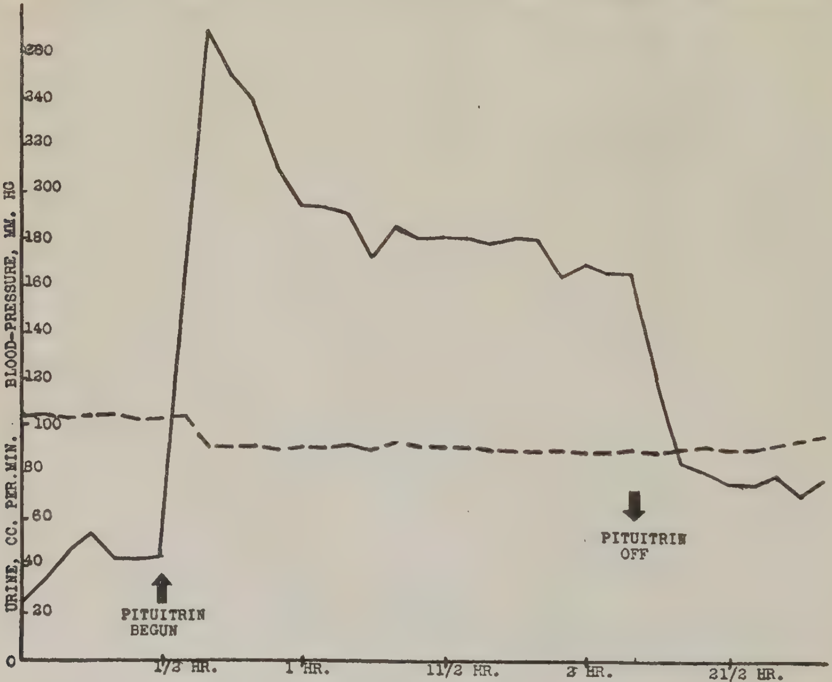

FIG. 1.

Ringer diuresis, injection rate 2.5 cc. per min.
Pituitrin, 1 cc. in 400 Ringer solution.

associated with gradual rise of blood pressure. Change to the solution with pituitrin added was made when the plateau was reached and had been maintained for from 15 to 30 minutes. Ringer solution, 5 per cent sodium chloride, 5 per cent and 10 per cent urea, and 0.5 per cent theocin solutions were used as the diuretic agents. With Ringer and sodium chloride solutions the urine flow was considerably less than the injection rate. With urea solutions the urine flow exceeded the injection rate, the animal being thus progressively dehydrated. The pituitrin was varied as to solution strength, duration of injection, and method of administration. Table I gives the results with pituitrin variously given. Table II gives the effects of repeat injections of pituitrin. Fig. 1 gives the detail of a typical experiment.

In all experiments without exception the addition of pituitrin to the solution injected increased the rate of urine flow. Effect of subcutaneous injection was not essentially different from the effect of intravenous administration (except that absorption time delayed the onset). There was no qualitative difference with dilution. Effects on urea and theocin diuresis were similar to effects on Ringer and 5 per cent sodium chloride solution. In nearly all instances the pituitrin effect had an immediate abrupt onset, rapidly rising to a maximum and slowly diminishing but remaining definitely above the previous level even though the pituitary injection was continued for an hour. On changing from the pituitrin solution to the original solution the rate of urine flow falls off although it usually maintains an average above that of the initial period. We have not been able to determine that it represents a definite antidiuretic period. (In this respect our results have differed from some of those previously reported.)

Summary. When diuresis is induced in anesthetized rabbits by the method of continuous intravenous injection pituitrin acts to augment such diuresis. The effects are similar with various diuretics and with varying dosage and administration of the pituitrin.

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Southern Branch.

Tulane University, May 19, 1927.

3610

Cardiac Output as Influenced by Ephedrin, Homocamfin, Quinidine, Quinine, Chloral and Chloroform.

CHAPMAN REYNOLDS AND S. N. BLACKBERG. (Introduced by J. T. Halsey.)

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Cardiac output per minute was determined in these experiments by the principle of Fick.[1] In most instances blood for analysis was obtained by right and left ventricular puncture, (see Bancroft, Boycott, Dunn and Peters,[2] and Marshall.[3]) In certain experiments, where chloroform was used, arterial blood was obtained from the femoral artery and venous blood through a cannula inserted into the right auricle through the external jugular vein, as suggested by Stewart[4] for experimental determination of cardiac output.

In all but two groups of experiments determinations were made on trained unanesthetized dogs. Homocamfin and ephedrine effects were also studied in dogs whose circulation had been altered by chloral and by chloroform. Blood pressure was determined by means of sphygmomanometer and stethoscope, except in the chloroform experiments, where the carotid artery as well as the trachea was opened.

Chloral Hydrate: Eight observations were made of the effect of toxic doses on normal dogs. In six of these the output was increased by 17 to 81 per cent (average 58), blood pressure decreased an average of 32 per cent, heart rate increased an average of 35 per cent, oxygen consumption remained practically unchanged. In two experiments the output decreased 6 and 53 per cent. For this we can offer no explanation, for in subsequent observations with the same dosage the same dogs showed an increase of 44 and 81 per cent in output. Average changes are plotted as Curve 1 in the chart.

Quinidine: In two normal dogs large doses of this drug increased the output 24 and 36 per cent, blood pressure rose 11 and 28 per cent, heart rate increased 43 and 15 per cent, while the consumption of oxygen was practically unchanged in one and diminished by 17 per cent in the other. Averages are indicated by Curve 2.

Quinine: In one observation on a normal dog a large dose increased the output 40 per cent and the blood pressure 19 per cent, while heart rate and oxygen consumption were practically unchanged. (Curve 3.)

Homocamfin (Cyclosal) on Normal: In three experiments the output was decreased from 7 to 44 per cent (average 20), blood pressure increased 11 to 23 per cent (average 18), heart rate decreased moderately once and increased slightly twice, averaging an increase of 3 per cent, oxygen consumption increased twice and decreased once, averaging an increase of 2 per cent. (Curve 4.)

Ephedrin on Normal: In two experiments the output diminished 25 and 29 per cent, blood pressure increased 30 and 33 per cent, heart rate decreased 4 and 23 per cent, oxygen consumption increased 6 per cent in each instance. (Curve 5.)

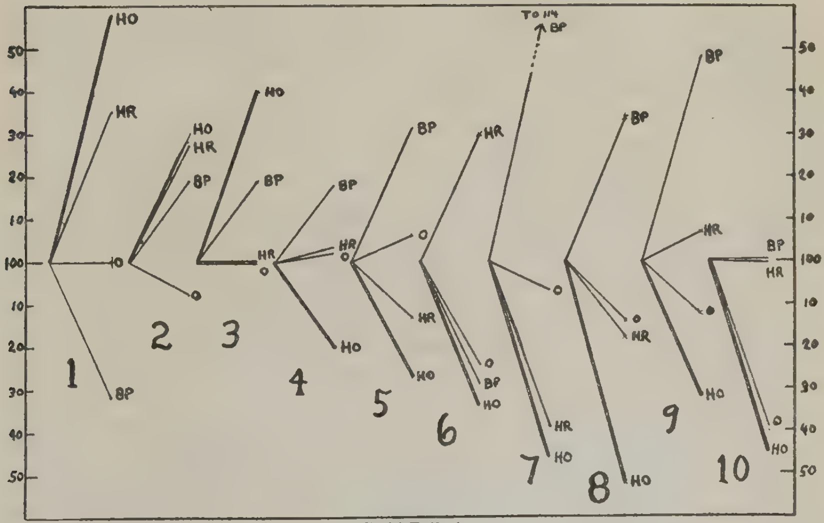

CHART 1.

Percentage Changes in Heart Output, Blood Pressure, Heart Rate, and Oxygen Consumption.

Curve 1: chloral hydrate on normal. Curve 2: quinidine on normal. Curve 3: quinine on normal. Curve 4: homocamfin on normal. Curve 5: ephedrin on normal. Curve 6: chloroform on normal. Curve 7: ephedrin following chloral hydrate. Curve 8: homocamfin following chloral hydrate. Curve 9: ephedrin following chloroform. Curve 10: homocamfin following chloroform.

Chloroform: In four experiments chloroform, in amounts not sufficient to induce muscular relaxation or abolition of reflexes, reduced the output 26 to 58 per cent (average 34), lowered blood pressure 23 to 33 per cent (average 29), increased heart rate 16 to 36 per cent (average 30); oxygen consumption was increased in one case 4 per cent, in all others reduced 20 to 45 per cent, averaging for all a reduction of 24 per cent. (Curve 6.) (This is in accord with the results of observations previously made by Blalock, as yet unpublished, but reported to us in a personal communication.)

Ephedrin after Choral: In three experiments in which choral had increased the heart output, ephedrin diminished the output 36 to 56 per cent (average 46), raised blood pressure 60 to 205 per cent (average 114), decreased the heart rate an average of 39 per cent and the oxygen consumption 6.5 per cent. (Curve 7.)

Homocamfin after Chloral: In four experiments in which the heart output had been increased by chloral, homocamfin diminished it 42 to 62 per cent (average 52), increased the blood pressure 18 to 42 per cent (average 34), decreased heart rate an average of 18 per cent, and decreased oxygen consumption an average of 14 per cent. (Curve 8.) In one experiment in which chloral had decreased the output 6 per cent, homocamfin increased it 28 per cent. In another in which chloral had decreased the output 53 per cent, homocamfin increased it 108 per cent.

Ephedrin after Chloroform: In three experiments in which the heart output had already been reduced by chloroform, ephedrin reduced it still further from 21 to 47 per cent (average 32), increased blood pressure 16 to 90 per cent (average 48), increased the heart rate twice and decreased it once, averaging an increase of 7 per cent; diminished oxygen consumption 6 to 20 per cent (average 12). (Curve 9.) In one experiment where the anesthesia was very light and the dog was constantly struggling, the heart output after ephedrin increased 42 per cent, although blood pressure and heart rate were increased as usual.

Homocamfin after Chloroform: In two experiments in which the heart output had been reduced by chloroform, homocamfin diminished it still further 43 and 50 per cent, while not changing blood pressure or heart rate materially, oxygen consumption decreased 36 and 44 per cent. (Curve 10.)

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3611

The Effects Produced in the Rabbit by Feeding Cultures of Monilia psilosis.

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From the Department of Pathology of the Tulane University School of Medicine.

From a human case of typical Sprue, cultures of the *Monilia psilosis* were isolated both from the oral lesions and from the stools. These cultures were identical in biological characteristics, and corresponded in every manner to those described by Ashford[1] in his experimental work on Sprue. The organism grows in chains, has a motile body in the vacuole, forms dense mycelia and ferments saccharose, maltose, and levulose. Pure cultures were obtained by plating an emulsion on Sabouraud's medium. Several other forms of yeast were also obtained from foamy stools which do not, however, conform culturally to the true *Monilia psilosis*. These were employed as culture suspensions in feeding experiments upon control animals.

Seventeen full grown rabbits were employed, six of which were used as controls. The *Monilia psilosis* was cultured for 10 days in a 4 per cent dextrose bouillon. Two of the rabbits were injected intravenously with 1.0 cc. of this culture, and one of these was also injected with 0.2 cc. sublingually. Both rabbits died in approximately 24 hours and from each a pure culture of the *Monilia* was recovered from the heart's blood, kidneys and liver. Through this procedure the pathogenicity of the organism for the rabbit was determined.

TABLE I.

Rabbit	Red Blood Count			White Blood Count		
	Initial count			Initial count		
1.	6,000,000	25th day 4,000,000	29th day 3,250,000	15,000	25th day 13,750	29th day 12,500
2.	4,750,000	32nd day 2,750,000	38th day 2,750,000	13,500	32nd day 14,000	38th day 14,250
3.	5,250,000	21st day 5,000,000	54th day 5,000,000	10,750	21st day 16,000	54th day 12,000
4.	4,500,000	5,000,000	38th day 4,750,000	8,000	10,500	38th day 9,000
6.	5,500,000	28th day 4,250,000	48th day 3,500,000	11,000	28th day 14,000	48th day 10,000
7.	4,750,000	50th day 4,000,000	57th day 3,750,000	13,000	50th day 16,000	57th day 18,000

Feeding Experiments: Nine rabbits were fed with the 10 day culture of this same strain of *Monilia* grown in 4 per cent dextrose bouillon. The cultures were administered daily with oats and carrots. Of these 9 rabbits, 6 died between the 31st and 61st day. The three remaining animals continued entirely normal

The blood counts of the six animals which became sick and died are shown in the table.

Rabbit 1: After the 25th day the animal showed loss in weight, became sluggish and refused food, grew weaker and died on the 31st day. Autopsy: Intestines are slightly distended, much mucus is present in the lower colon, the mucous membrane appears normal. The spleen is small and dark red. All other structures appear normal. Abnormal extensive deposits of blood pigment are in the spleen and to less extent in the liver. The bone marrow shows increase in the red portions, but no microscopic change.

Rabbit 2: After 32 days the animal refused food, became droopy and lost weight, until death on the 39th day. Autopsy: the bowel slightly distended with gas, with very small amount of mucus in the colon. The spleen small and dark red color. Marked deposit of blood pigment in spleen and liver.

Rabbit 3: Apparently normal for 3 weeks, then stools became periodically loose, watery, but at no time gave the true buoyant, foamy sprue stool. The appetite became poor, animal lost weight, grew progressively weaker, and died on the 56th day. At autopsy the gross findings were negative. The microscopic study showed acute cholangitis, and a chronic parenchymatous nephritis, with marked evidence of calcium deposit in the epithelial lining of the loops of the Henle. No pigment deposits were noted.

Rabbit 4: Animal normal until 4th week, when it suddenly refused food, lost weight, grew weak, and died on the 41st day. At autopsy the liver was of a light reddish brown, the spleen normal, fatty degeneration of the kidney epithelium marked. Occasional small deposits of blood pigment present in the spleen.

Rabbit 6: Loose stools developed on the 28th and 48th day, but not sprue-like in character, nor did they show fat crystals. A week prior to death, on the 50th day, there was loss of appetite, emaciation and weakness. The autopsy showed bright red spleen, normal, slightly softer in consistency. Massive amounts of blood pigment in the spleen, both intra and extra-cellular and arranged in very large aggregations, scattered throughout the organ. In the liver a lesser amount of pigment appeared both inside and outside of the intralobular sinusoids. The kidney showed cloudy swelling and some fatty degeneration.

Rabbit 7: Died on the 62nd day, having shown signs of loss of weight and appetite from the 50th day on. Microscopic study showed considerable blood pigment deposited in the spleen, none in the liver. In the kidney there was a slight cloudy swelling, and some fatty degeneration. Cultures from the intestinal tract of all six animals at post mortem yielded growths of *Monilia psilosis* while those made from the heart's blood, liver, spleen and kidney were negative.

Controls: Six rabbits were treated in a similar manner as those of the *Monilia* experiments, excepting that bouillon growth of the other saccharomycetes isolated from the stools of the same sprue patient was the culture employed. These animals, although fed over a period of approximately 61 days with these presumably saprophytic cultures remained in every way normal.

The experiments indicated that the *Monilia psilosis* is pathogenic for rabbits when injected intravenously and also when given by feeding. The clinical picture produced in the animals of the feeding experiments is quite constant although no specific syndrome corresponding to clinical sprue occurred. The only manifestations relating to this clinical entity which were produced were loss of weight and some anemia. The striking feature of the stool character and the stomatitis of human sprue were in no instance noted. From the pathological standpoint the almost constant evidences of extensive blood destruction as shown by the marked deposits of blood pigments in the various organs, especially in the spleen, were of striking interest.

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[1] Ashford, D. K., *Am. J. Med. Sc.*, 1917, cliv, 157.

3612

Variations of Blood Inorganic Phosphorus With Exercise.

ARTHUR O. KASTLER. (Introduced by W. Denis.)

From the Department of Biochemistry of the School of Medicine, Tulane University.

With regard to the relationship apparently existing between carbohydrate metabolism and the metabolism of phosphorus, it was proposed to study the behavior of the inorganic and the acid-soluble phosphorus in the blood of dogs whose carbohydrate supply was being rapidly utilized by strenuous exercise. The glycogen reserve of one series of animals was depleted by a preliminary 48 hour starvation.

The dogs were trained to run freely on a treadmill whose belt speed was 450 feet per minute. Rest periods at varying intervals during the exercise permitted the drawing of blood samples from the heart, and allowed the animals to regain their normal breathing. The total actual exercise varied from 30 minutes to 240 minutes.

The results from 10 experiments on 3 dogs showed:

1. Inorganic phosphorus in the blood of starved dogs taking standard exercise, at first decreased considerably (65 per cent in 90 minutes exercise), and then with continued exercise, rose towards the normal. During recovery, inorganic blood phosphorus rose to values approximately 60 per cent above normal. Total acid-soluble phosphorus of the blood paralleled its inorganic fraction during exercise and recovery. Sugar, calcium, and the percentage of corpuscles in the blood remained relatively constant.
2. Dogs that had not been starved showed very much less marked blood phosphorus changes with exercise. (30 per cent initial decrease in 60 min. exercise.)
3. A given duration of exercise produced a definite inorganic phosphate concentration in the blood.
4. Changes in the concentration of blood inorganic phosphate during recovery from exercise were independent of the duration of exercise.

This is a preliminary report.

3613

Scarlatinal Nephritis Experimentally Induced in the Dog.*

CHARLES W. DUVAL AND R. J. HIBBARD.

From the Department of Pathology, Tulane School of Medicine.

Since the publication[1] of the results of our studies upon experimental nephritis in the rabbit with the toxic principle of *Streptococcus scarlatinae,* we have found that the dog is a more satisfactory animal for the purpose of causing nephritis with this particular injurious agent. This animal has proven to be not only highly susceptible to infection but developed regularly a severe and often fatal form of acute glomerulonephritis following the injection of the specific streptococcal toxin alone. None of the animals showed at any time an exanthem; however, it is significant that the induced

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* Aided by a Grant from the David Schwartz Research Fund of Tulane Univ.

kidney lesions completely correspond histopathologically to the glomerular and acute interstitial scarlatinal nephritis of man.

This communication is a brief report of the effects upon the kidneys of the dog produced by the viable culture and also the pure toxin of *Streptococcus scarlatinae.*

All dogs employed in the experiment were healthy and under three years of age. The animals were kept under close observation for a period of ten days prior to inoculation, during which time daily analysis of the urine was made and the blood chemistry determined. Only dogs that showed normal kidney function throughout this period were selected for the experiment. The preliminary study of the urine and blood was carried out by our colleague, Professor Denis, of the department of biochemistry.

Infectivity of the Dog. Six full-grown, healthy dogs were inoculated with living cultures of *Scarlatinal streptococcus.* Two animals received the injection intravenously, two subcutaneously and two intraperitoneally. In each instance the dosage was 10 mills of the surface growth from slanted sheep-serum agar which had been washed off and suspended in 50 mils of sterile normal saline. All animals of the series promptly developed a generalized infection and died in 2 to 5 days following the inoculation. Daily urine examination showed pus, blood, albumin and casts. At autopsy there was revealed the usual signs of sepsis, and in addition a markedly acute hemorrhagic nephritis.

Toxin Effects upon the Kidney of the Dog. Eight young, healthy dogs were injected intravenously with 5 mils each of filtered streptococcal "lysate" which had been prepared *in vivo* after the method described by us in a previous publication.[2] The animals developed within 4 to 6 hours symptoms of toxemia, and 24 hours later were extremely ill. The urine macroscopically was bloody and analysis showed quantities of albumin, granular casts, bile and blood. Two of the animals died on the fourth and 3 on the fifth day following the inoculation. The others survived, and though apparently well have continued to show, at intervals, albumin and casts in the urine. Two months later these survivors were sacrificed and the kidneys revealed varying degrees of chronic diffuse nephritis.

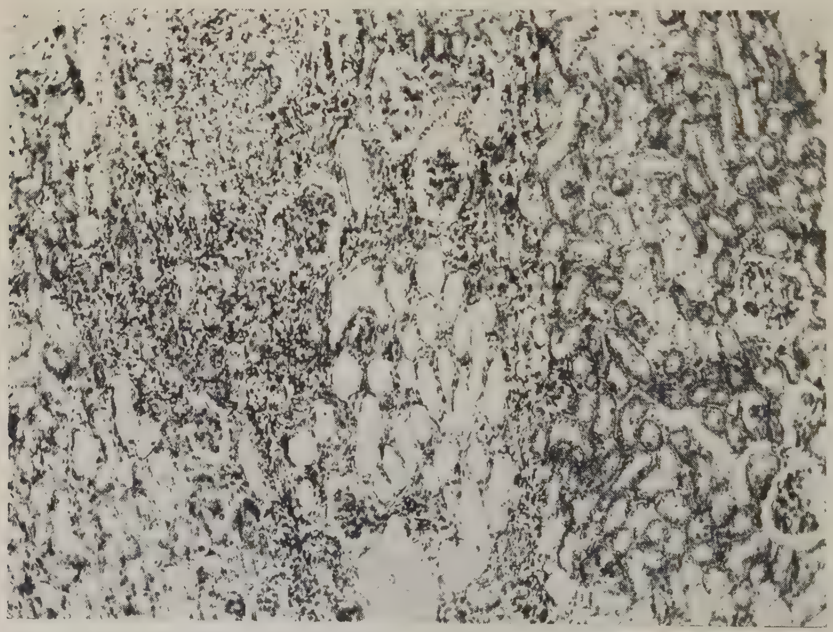
The Kidney Pathology Induced in the Dog. Constant and significant structural changes occur in the kidney of the experimentally produced nephritis with *Streptococcus scarlatinae.* The lesions are primarily glomerular or interstitial, the determining factor depending upon the character of the material employed. For example, the glomeruli are first affected where the toxic principle alone is used, and regardless of whether the injection is made intravenously or subcutaneously. On the other hand the primary interstitial lesion occurs in the animals inoculated with the living culture, and in which a generalized infection has resulted.

The acute interstitial lesion is a focal infiltration of the intertubular tissues with cells of the lymphocytic variety. As a rule the neutraphiles are absent or only few in number; however, in some of the more advanced lesions they occur but are not as numerous as the lymphoid and plasma cells. Associated with the interstitial lesion are viable streptococci which are readily demonstrable in stained sections and recoverable in pure culture from the fresh tissues.

The absence of fibroblasts or any other evidence of stromal activity in the early interstitial lesion is of significance. This fact clearly indicates that the lymphocytic infiltration is a true reaction on the part of the host to the injurious agent and in no sense reparatory, from which it may be inferred that acute interstitial scarlatinal nephritis of man is the same kind of reaction.

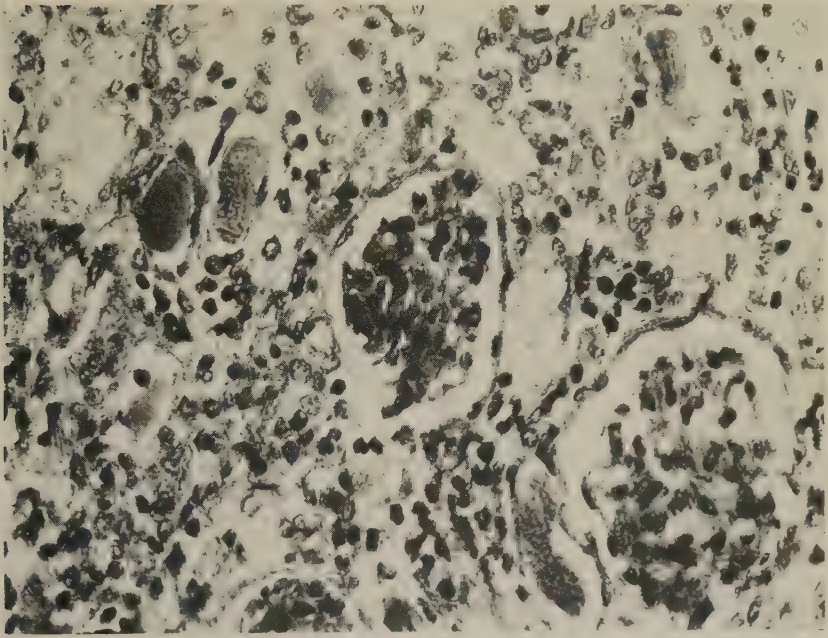
In regard to the primary glomerular lesion in the dog, the streptococcal toxin affects especially the capillaries of the kidney tufts, producing various alterations in the whole glomerular structure.

FIG. 1.



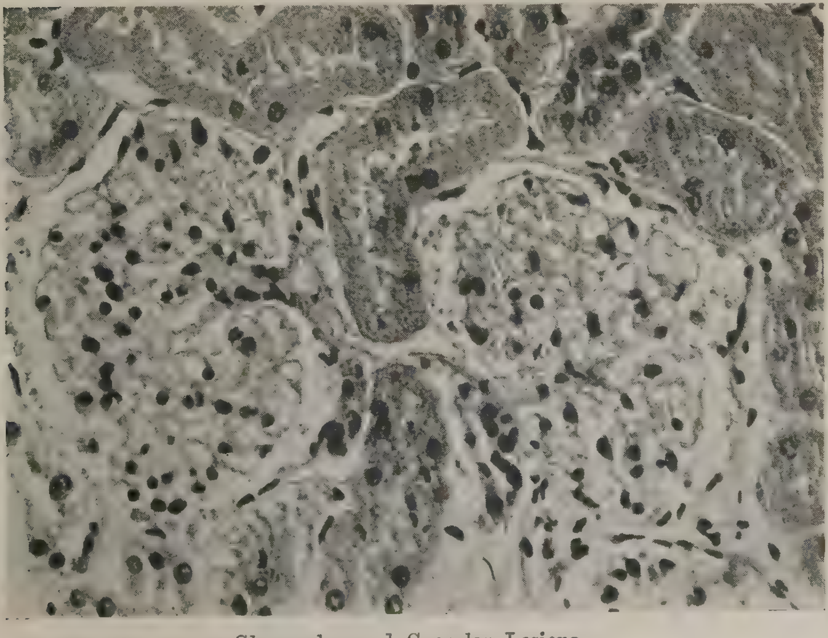
Acute Interstitial, Non-suppurative (induced by the injection of specific culture).

FIG. 2.



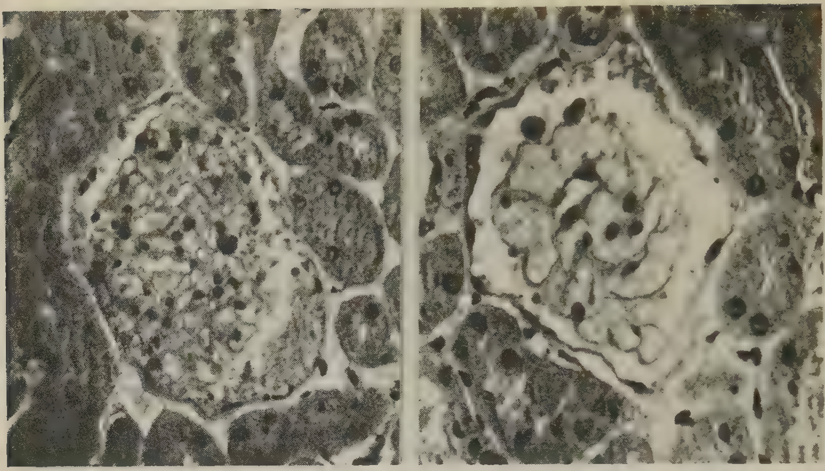
Acute Interstitial, Non-suppurative (induced by the injection of specific culture).

FIG. 3.



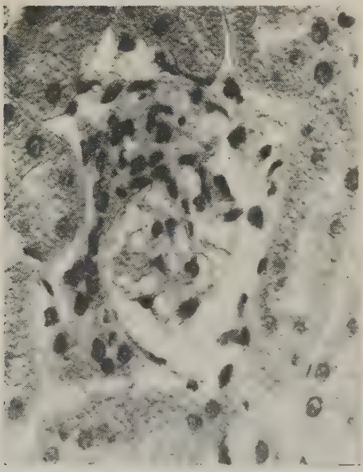
Glomerular and Capsular Lesions.

FIG. 4.



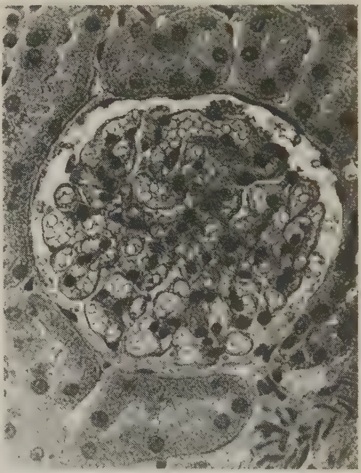
Glomerular Lesion. Degeneration of Tuft.

FIG. 5.



Glomerular and Capsular Lesions.
(Early epithelial "crescent" formation.)

FIG. 6.



Early Glomerular Lesion.
(Intracapillary dilatation and engorgement.)

There are lesions in the wall of the capillaries which cause the formation of thrombi, in consequence of which the vascular loops become greatly dilated, occluded and later adherent to Bowman's capsule. Other glomerular tufts become enlarged through the appearance of quantities of endothelial cells in the lumina of the capillaries.

Bowman's capsular spaces generally contain blood in the form of hyalin masses, also albuminous material and desquamated epithelium. Later, in those capsular spaces where hemorrhage has occurred, early epithelial proliferation ("crescent" formation) is noted. All these structural changes cause the glomeruli to undergo further and more serious alteration through replacement by fibrous tissue of the destroyed capillary loops.

While tubular changes are not an early feature in either the glomerular or interstitial type of experimental scarlatinal nephritis, they occur later in the process and appear in the form of epithelial degeneration, especially of the convoluted portion of the tubules. Here the lining cells become swollen through the presence of fluid, granules, fat and hyalin droplets. Often the lumen is filled with blood, desquamated epithelium, granular and hyalin casts.

The acute lesions of the experimental nephritis in the dog are recognized macroscopically by an increase in size of the kidney, by swelling of the glomeruli which project above the cut surface, and by pinhead and smaller discrete yellowish white foci in the cortical substance and by sprinkling throughout the organ with hemorrhages.

Summary. The various types of glomerular and also the acute interstitial nephritis of human scarlet fever can be regularly produced in the dog with the culture and with the pure toxic principle of *Streptococcus scarlatinae.*

The experimentally induced acute nephritic lesions are of two forms, glomerular and interstitial, and these are alike in kind and variety to the acute scarlatinal nephritis of man.

The complete analogy of the experimental nephritic lesion in the dog to that in man is of unusual interest since it affords the opportunity to study the acute lesions in the order of their related sequence and is a means of tracing the changes that lead to a progressive diffuse nephritis.

This is a preliminary report.

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[1] Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1926, xliv, 567.

[2] Duval, C. W., and Hibbard, R. J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 850.

3614

The Effect of Carbon Arc Radiation on the Blood of Dogs.

H. S. MAYERSON. (Introduced by Henry Laurens.)

From the Laboratory of Physiology, Tulane University School of Medicine.

As a preliminary to the investigation of the influence of radiation on anemia, three normal adult dogs (white, short haired) were exposed to known amounts of flaming carbon arc radiation. The first was irradiated abdominally for 45 minutes at 1 M (45 g. cal. per sq. cm.) and the dosage progressively increased to 1 hour at 60 cm. (166 g. cal. per sq. cm.) during a period of 13 daily exposures. The other 2 were given 4 massive abdominal irradiations (88 to 124 g. cal. per sq. cm.) at intervals of 2, 3 and 6 days, respectively. The results are of the same general nature in all.

The primary result of an individual exposure is to increase blood volume by about 30 per cent with recovery to normal by the afternoon of the same day, the increase being principally due to an increase in plasma volume. With subsequent exposures, red cell number and volume and hemoglobin increase approximately 10 per cent. Platelets show a consistent drop in number and volume during irradiation with recovery to normal after each exposure, and serve as an index of dilution. The increase in red cells was maintained in the first 2 experiments, the value in one dog being 15 per cent above normal 6 weeks after the last exposure. Hemoglobin shows a slight increase (3 per cent) during the post-irradiation period in experiment 2, but decreases 11 and 7 per cent, respectively, in the others. The average hematocrit value for the post-irradiation period in the first dog is about 12 per cent higher than the pre-irradiation level; it is 9 and 5 per cent lower, respectively, in the last 2 experiments. A consideration of the color, volume and saturation indices shows that the new red cells being formed in this period are smaller in size than normal, saturation in experiments 2 and 3 being normal, in experiment 1 only 84 per cent. In experiments 1 and 3 there is a post-irradiation rise in platelets of 4 and 15 per cent, respectively, persisting for about 10 days. The leucocytes fluctuate during the irradiation period, but ultimately decrease to markedly lower levels, the values in the first 2 experiments being only 50 per cent normal 1 week and 4 weeks, respectively, after the last exposure.

This is a preliminary report.

3615

Duration of Electrogram and of Mechanical Response of Turtle Ventricle.

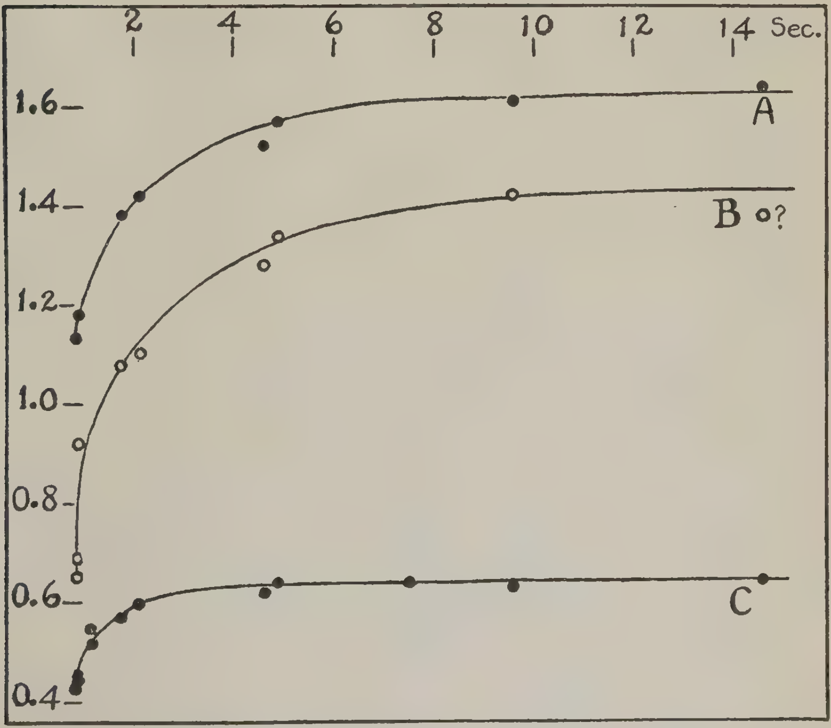
ROBERTA HAFKESBRING AND RICHARD ASHMAN.

From the Department of Physiology, School of Medicine, Tulane University.

The effect of varying rates and volumes upon the duration of the phases of the isometric contractions of the ventricle has frequently been investigated. The influence of these factors upon the duration of the electrogram, and particularly the correlation of mechanical and electrical changes, have not been so fully determined. It is with this correlation, and with certain effects of fatigue, that this abstract deals.

The excised turtle ventricle was perfused with slightly alkaline

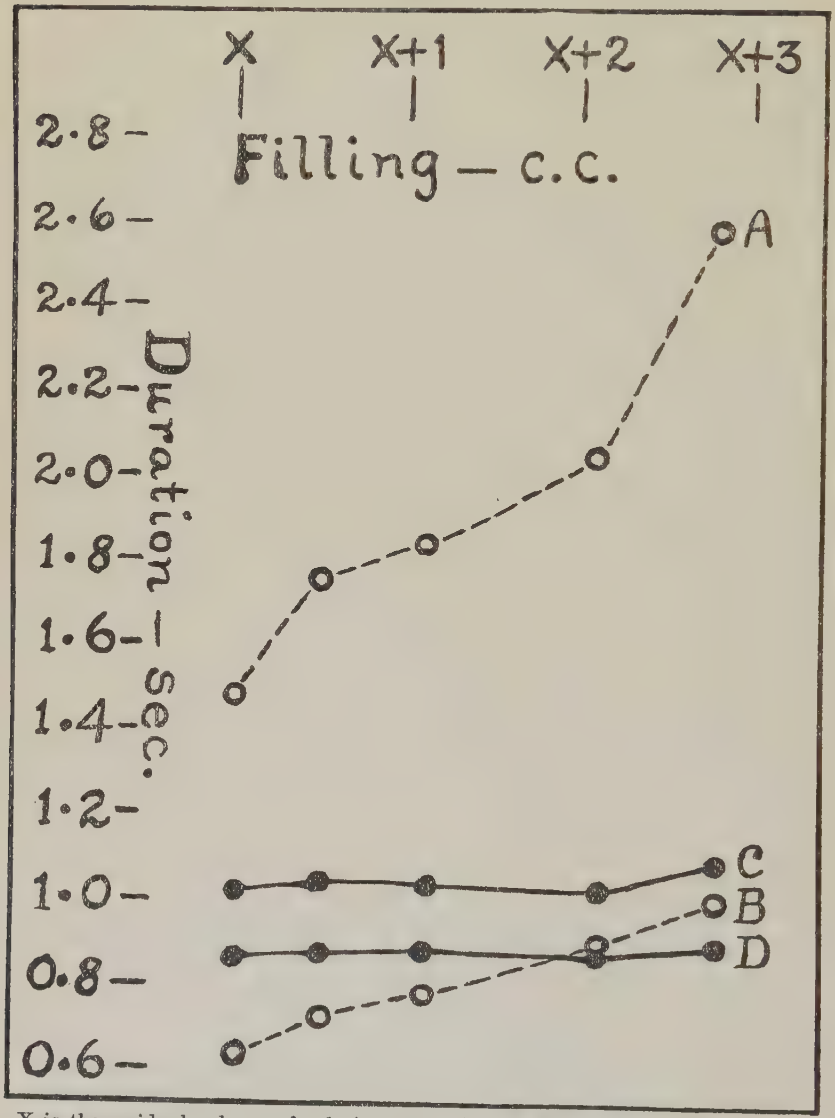
FIG. 1.



Abscissae, cycle lengths; ordinates, duration of the phase. A, total mechanical response; B, electrogram (R to end of T); C, phase of rising tension. Some evidence of fatigue is present.

(pH 7.5—7.8), well-oxygenated and strongly buffered Ringer's solution. During temporary suspension of perfusion, the isometric responses to single induction shocks at varying rates were recorded. Both mechanical and electrical responses were simultaneously photographed on the electro-cardiographic film.

FIG. 2.



X is the residual volume of solution remaining in the heart which has emptied itself; A, total mechanical response; B, phase of rising tension; C, electrogram (R to end of T); D, electrogram (apex of R to apex of T).

As illustrated in figure 1, the total duration of the electrical response (measured from the apex of the R-wave to the end of the T) and the duration of the phases of rising and falling tension within the ventricle are similarly influenced by changes in cycle length (rate).

In contrast are the effects of filling (fiber length) upon the durations of the electrical and mechanical responses. Contrary to previous report (turtle),[1] we find that increased filling, up to the limits of ventricular capacity, prolong the electrical response only very slightly or not at all.* The phases of rising and falling tension, on the other hand, increase with augmented filling (figure 2).

Fatigue has very striking effects. When the perfusion is inadequate and recovery processes incomplete there is with activity a progressive decrease in the duration of the electrogram and of the phases of rising and falling tension. With prolonged rest nearly complete recovery may occur.

When effects of rate change are studied in the unfatigued heart the maximum duration of the electrical and mechanical responses may be attained if the stimulus is applied as soon as relaxation is complete. Depending upon the degree of fatigue, these phases may remain shortened when the stimulus is applied long after the end of relaxation. The extent of relaxation is not, therefore, a factor controlling the duration of the response.

Our results indicate, we believe, that the important factor determining the duration of the electrogram is the chemical condition or degree of recovery of the muscle at the instant the stimulus is applied. The degree of filling, on the contrary, as stated above, influences the duration of the electrogram very slightly, if at all. The mechanical phases, on the other hand, are determined not only by the degree of recovery, but also by the extent of filling of the ventricle.

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* In one or two instances a slight increase is indicated by our data. Generally, however, any change which may have occurred was within the limits of experimental error.

[1] Daly, I. de B., *Proc. of Royal Soc.*, 1923, B 95, 279.

Missouri Branch.

Washington University, May 18, 1927.

3616

Comparative Studies of Histological Skin Changes Produced by X-Rays and by Lipoid Solvents

LOUIS H. JORSTAD AND CLINTON W. LANE. (Introduced by M. T. Burrows.)

From the Barnard Free Skin and Cancer Hospital, the Department of Surgery, Washington University School of Medicine.

By varying the time period of exposure and transmitting the ray through an opening 1 cm. in diameter, we have produced various degrees of x-ray burns of the skin in a series of white rats. The quantity of x-rays used varied from 5 miliampere minutes to 50 miliampere minutes. Animals were placed on high vitamin A, low vitamin A, and balanced stock rations to determine if these dietaries would produce a difference in reaction to the x-rays. Our studies, thus far, have not shown any striking differences. The characteristic gross picture is depilation over the area of skin exposed, with the persistence of a roughened, scarred skin that appears from 14 to 21 days after the exposure to the x-rays. Five miliampere minutes and 10 miliampere minutes produce no effect grossly apparent.

Microscopically the lesion is that of shrinking and edema of the epithelial layer, hyperplasia of the hair follicles, and in some cases a metaplasia of the basal layer of the skin. The subcutis shows varying degrees of hyalinization. With the high doses (50 miliampere minutes) there is a hyalinization of the subcutis and the glandular structures in it. The epithelium remains intact over the area, the higher dosages producing a pyknosis of the cell nuclei and a hyalinization of the cytoplasm.

In the second series we applied a 50 per cent coal tar or pine tar preparation to a denuded area 2 cm. in diameter. The white rat was used. The tar was painted on the area with a cotton swab every 2 or 3 days. A roughening of the skin and a gradual loss of hair was noted grossly. Microscopically a hyperkeratosis begins at the 30th day, and gradually progresses. At 45 days there is an extensive hyperkeratosis that in some cases involves nearly the entire epithelial layer, with an extension into the hair follicles and glands of the skin.

In comparing the effect of x-rays and the tar preparations in these cases it is interesting to note that the x-rays penetrate deeply to effect the corium and structures in it, while the tar preparations act locally at the site of application. Otherwise the effect of the two are similar.

Former studies from the laboratory show that coal tar, mazola oil, mineral oil, etc., may be classed as lipoid solvents.[1] When they are introduced into the subcutaneous tissue they remove the ergusia (lipoid element) from the tissues with which they come in contact. Further studies from the laboratory have shown that animals on a vitamin A deficient diet are protected for a time by certain doses of x-rays.[2] This indicates that x-rays must liberate this vitamin from the tissue absorbing it.

This is a preliminary report.

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[1] Jorstad, L. H., *J. Can. Res.*, 1926, x, No. 2.

[2] Burrows, M. T., Jorstad, L. H., and Ernst, E. C., *J. Am. Med. Assn.*, 1926, lxxxvii, 86-89.

3617

Further Study of the Bacteriemia of Experimental Streptococcus Endocarditis.

RALPH A. KINSELLA and C. M. HAYES.

From the Department of Internal Medicine, St. Louis University School of Medicine.

When the aortic or mitral valves of dogs are injured by passing a suitable steel rod down the left carotid artery, the resulting wound of the valve shows, at the end of 49 days, a hyalin-like mass at the point of injury. As late as 60 days after such injury the injection intravenously, of non-hemolytic streptococci results, constantly, in a bacterial vegetative growth on the injured area productive of petechiae, infarctions, thrombo-glomerular nephritis, and small areas in the heart muscle in which a round cell infiltration follows a punctate hemorrhage.

In four dogs the healing of the injury was not altered by the injection either before or after injury, of egg albumin, followed in three weeks by a second injection, which was in turn made three days before autopsy.

A strain of non-hemolytic streptococcus isolated from the blood of a patient with acute rheumatic fever, and having some virulence for white mice, and a strain from a normal throat having no virulence for mice, were equally successful in producing the infection of the injured valve.

Subacute bacterial endocarditis in humans is characterized by the presence in the blood stream of avirulent non-hemolytic streptococci. These streptococci are said to be avirulent from the standpoint of the action on white mice. They may or may not have been virulent before infecting the human subject.

Cultures of the strain from acute rheumatic fever, recovered from dogs with endocarditis produced as above, failed to infect fresh animals in two instances. A culture of the strain from a normal throat after such passage through a dog failed to infect a fresh animal which had had a valvular injury. Furthermore two different strains from clinical cases failed to infect animals whose valves had previously been injured.

In 21 dogs the bacteriemia, following the establishment of endocarditis, was of a type in which the number of colonies per cubic centimeter of blood rapidly increased to 300 on the fourth day after injection and countless by the 12th. This resembles an acute fatal bacteriemia in man.

In subacute endocarditis, in man, the colony-count remains uniform throughout the disease at a level which may be high or low. In 2 animals in which the strain from acute rheumatic fever was used, and in 2 animals in which the strain from a normal throat was used, the bacteriemia remained for 12 days at a low level, varying between 5 and 20 colonies per cubic centimeter of blood, by the serum of previously infected dogs.

In these experiments the longest duration of life was 31 days, the average, 18.

In 2 animals the inoculation of killed cultures of avirulent non-hemolytic streptococcus, up to the production of strongly agglutinating serum, did not prevent the easy subsequent infection of the injured valves when living cultures were injected.

Conclusions. Lack of virulence in strain from subacute bacterial endocarditis is a characteristic most probably acquired by passage through the host.

Agglutinated streptococci produce a type of bacteriemia in dogs with injured valves which simulates the bacteriemia found in human cases of subacute bacterial endocarditis.

3618

Filtrates From Scarlet Fever and Surgical Hemolytic Streptococcus Infections.

RALPH A. KINSELLA, O. GARCIA AND J. WADE.

From St. Mary's Hospital, St. Louis University School of Medicine.

In scarlet fever it is held that there is a special strain of hemolytic streptococcus responsible for scarlet fever. Nevertheless, by agglutination tests, all strains isolated from cases of scarlet fever are not identical. It has not been argued that a special phase of reactivity on the part of the host can lead to the clinical picture of scarlet fever after infection by any hemolytic streptococcus.

The criteria for investigating the specific nature of a given strain of hemolytic streptococcus, whether from scarlet fever or from other clinical infections, have been the preparation of a filtrate from special culture medium and the testing of such filtrate for its resistance to heating, its capacity to produce a dermal reaction in a susceptible subject, and its neutralization by anti-scarlet fever serum in skin tests.

The following would indicate either that these criteria are not sufficient to establish the specific qualities of a given strain, or that the phase of reactivity of a patient is important in determining whether infection by hemolytic streptococcus will result in scarlet fever or some other clinical picture. The filtrates were studied from the throat cultures of three cases of scarlet fever, two of which were caused by infection of wounds; and of three cases of bacteriemia following operations, in one of which the attending nurse developed scarlet fever.

In the three cases of scarlet fever the cultures from the throat failed to yield hemolytic streptococcus. In two of these cases, one of which was surgical scarlet and the other the usual variety, the predominating organisms were non-hemolytic streptococcus which were immediately used to infect filtrate broth. Subcultures of this broth failed to show any colonies of hemolytic streptococcus on blood agar plates. Many other cases of scarlet fever at the Isolation Hospital were studied, but all showed rich throat cultures of hemolytic streptococcus. Filtrates from the two cultures of non-hemolytic streptococcus were thermolabile, gave positive skin reactions in the arms of two Dick-positive subjects and these reactions were neutralized by Dick anti-toxin.

Of the three cases of hemolytic streptococcus infectoin, not scarlet fever, there was bacteriemia arising from mastoiditis in two instances, and from infected hemorrhoids in the other. In one of the cases of mastoiditis, a young adult, there was no history of previous scarlet fever.

In these three cases filtrates made from strains isolated from blood cultures were thermolabile, gave positive skin tests in two Dick-positive subjects, and the tests were prevented by Dick anti-toxin.

The meaning of these observations can be determined only when anti-toxin serum is produced in suitable animals, using the filtrates above, and the value of such anti-toxin tested in scarlet fever.

This is a preliminary report.

3619

Lactic Acid Formation in Muscle Extracts.

H. A. DAVENPORT AND MARGHERITA COTONIO.
(Introduced by P. A. Shaffer.)

From the Department of Biochemistry, Washington University School of Medicine.

Embden and Haymann[1] demonstrated that the addition of glycogen and sodium fluoride to freshly prepared muscle press-juice caused a very marked decrease in the inorganic phosphate which was present in the extract. Sugars were inactive in the reaction. The disappearance of the inorganic phosphate was interpreted as an esterification process in which hexose phosphate (lactacidogen) was formed. Meyerhof[2] showed that there is a production of lactic acid during the course of glycogen degradation by muscle extracts, and that the phosphates appear to take an active part in the changes involved. Fluoride caused a decrease in the production of lactic acid as well as a disappearance of the inorganic phosphates.

The fate of the added glycogen with reference to the amount of total hydrolysable carbohydrate present in the working mixtures at the end of the incubation seems not to have been studied.

In our experiments we have endeavored to balance the carbohydrate loss against the lactic acid gain, and, at the same time, form some conception of the amount of carbohydrate that took part in the esterification with phosphoric acid. Simultaneous determinations of lactic acid, free sugar, total carbohydrate, and inorganic phosphate were made at 0, 30, 60, 120, and 240 minute intervals on aliquot portions of mixtures of muscle extract and glycogen solution.

The extract was prepared from chopped rabbit muscle by mixing two parts of ice cold distilled water with one part of muscle and straining through muslin after allowing the mixture to stand one-half hour at 0°. The animals were anesthetized with amytal, to prevent the rise of lactic acid in the muscle, by abolishing the death struggles, and killed by bleeding. The extract was used immediately after its preparation.

TABLE I.

Exp. No.	Substances added	1. Lactic acid gain, mg.	2. H_3 PO_4 change mg.	3. Carbohydrate loss, mg.	4. Carbohydrate loss as mm. glucose	5. ½ L. A. gain + H_3PO_4 loss, mm.	6. X
1.	None, control	4.5	+2	3	0.016	0.005	—
	Glycogen	11.2	—7	21	.117	.132	1.3
	Phosphate	5.0	+1	3	.016	.018	—
	Glycogen and phosphate	13.7	—11	33	.183	.188	1.0
2.	None	1.2	0	1	.005	.007	—
	Glycogen	10.6	—7	18	.100	.129	1.7
	Glyc. and Phos.	17.8	0	17	.094	.099	—
3.	None	5.0	+1.6	4	.022	.012	—
	Glycogen	14.8	—10	32	.178	.183	1.0
	NaF	—0.6	—1.5	0	0	.012	—
	Glyc. and NaF	0.4	—21	25	.139	.216	1.5

Table I shows the changes which had occurred in 15 cc. of muscle extract after 4 hours incubation at room temperature. The free sugar is included in the total carbohydrate.

A comparison of columns 4 and 5 shows that the carbohydrate loss parallels the algebraic sum of the lactic acid gain and inorganic phosphate loss. The esterification of carbohydrate would account for the phosphate loss, but the carbohydrate loss can be explained only by assuming that little or no reducing sugar is recovered by acid hydrolysis of the ester. This is likely, for Furth and Marian[3] have shown that only one-third of the theoretical glucose reducing value is obtained by hydrolysis of yeast hexose phosphate.

If the total carbohydrate loss (C) be assumed to be due to conversion to lactic acid (LA) and to hexose phosphate (HP), then $C=LA+HP$, and the equation $C=LA/2+x(H_3PO_4)$ (each quantity being expressed in mMols.) should indicate the molar ratio of phosphoric acid to hexose in the hexose phosphate. Column 6 gives the values for X in the experiments in which glycogen was added to the extract. It seems significant that these values lie between 1 and 2, and a logical explanation would be that a mixture of mono and diphosphate was formed.

The results support the hypothesis that glycogen passes through a carbohydrate-phosphate complex when it is transformed into lactic acid in muscle, and confirm the observations of Embden[1] and Meyerhof.[2]

This is a preliminary report.

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[1] Embden, Gus., and Haymann, Clare, *Zt. f. Physiol. Chem.*, 1924, cxxxvii, 154.

[2] Meyerhof, Otto, *Biochem. Zt.*, 1926, clxxviii, 395, 462.

[3] Furth, O., and Marian, J., *Biochem. Zt.*, 1926, clxvii, 123.

3620

Observations on Glyoxals, Their Determination and Behavior.

NOBORU ARIYAMA. (Introduced by P. A. Shaffer.)

From the Department of Biological Chemistry, Washington University School of Medicine.

Shaffer[1] observed that the addition of alkali cyanide in small amounts to glyoxal produces in slightly alkaline solution a very highly reducing substance, which rapidly reduces many dyes and other reagents; and he suggested that this reaction be utilized for a quantitative method for the determination of glyoxals. Among the reagents which develop color on reduction are the complex phosphotungstic acids, and of these the arseno phospho-tungstic reagent used by Benedict[2] for uric acid was found to be well suited for our purpose. Without cyanide, glyoxal or methyl glyoxal scarcely reduces this reagent, but with cyanide reduction is rapid and proportional to the glyoxal present. Six hundredths mg. or more glyoxal may thus be determined with an accuracy of ±5 per cent. To the glyoxal solution (preferably about 0.6 mg.) are added 2 cc. of Benedict's reagent, followed by 1 cc. of M NaCN and 5 cc. of M Na_2CO_3 in the order named. A similar mixture is made with 10 cc. 0.001 M glyoxal (0.58 mg.) as a standard. After 10 minutes at room temperature the mixtures are diluted to 100 cc. and read by colorimeter.

The notable effect of cyanide, in markedly increasing the reducing power, is not limited to glyoxals, having been observed also with uric acid and with cystin, but is exceptionally striking with the glyoxals.

With this method it is possible to follow the transformation of glyoxals by alkali, by tissue extracts (glyoxalase) and by cyanides. The following observations have been made. In pure buffer solutions at pH below 7 to 8 glyoxal and methyl glyoxal are quite stable; at higher pH the conversion to the corresponding hydroxy acid occurs, becoming rapid and complete at pH 12.5. At intermediate reactions at which the conversion is relatively slow, less lactic acid appears, then corresponds to the methyl glyoxal destroyed.

A comparison of the relative glyoxalase activity of filtered 20 per cent water extracts of various tissues on added methyl glyoxal shows that liver is most active, brain, skeletal muscle, heart muscle, lung, spleen, kidney and blood being about half as active. Simultaneous determinations of glyoxal and of lactic acid show that under the influence of tissue extracts methyl glyoxal is quantitatively converted to lactic acid. The optimum pH concentration for the glyoxalase activity is pH 7.0.

Meyerhof[3] has stated that dilute hydrocyanic acid converts methyl glyoxal to lactic acid. Our observations are in conflict with this statement. The action of cyanide is catalytic, a concentration of 10^{-5} M converting a much larger amount of methyl glyoxal rapidly in neutral buffer (where the glyoxal without cyanide is stable); but the product is not lactic acid. An acid is formed in amount about half the theoretical. The acid is volatile, is neither formic nor acetic nor pyruvic, but its identity is not established. The reactions by which methyl glyoxal is transformed under the catalytic influence of cyanide and by alkali or tissue extracts are therefore quite different. With cyanide a very much higher reducing intensity is attained than with either alkali or tissue extracts.

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[1] Shaffer, P. A., unpublished.

[2] Benedict, S. R., *J. Biol. Chem.*, 1922, li, 187.

[3] Meyerhof, O., *Biochem. Z.*, 1925, clix, 432.

3621

The Inhibiting Influence of Formaldehyde Upon the Dale Reaction.

A. I. KENDALL AND H. L. ALEXANDER.

From the Department of Bacteriology, and the Department of Internal Medicine, Washington University School of Medicine.

Kendall[1] has shown recently that formaldehyde will reduce smooth muscle contraction that has been initiated by histamin and by a histamin-like substance obtained in filtrates from cultures of *B. welchi.* Inasmuch as one theory of smooth muscle contraction induced by anaphylaxis postulates the liberation of some substance resembling histamin, the effect of formaldehyde on such contraction was studied.

Female guinea pigs weighing about 250 gm. were injected intraperitoneally with 0.2 cc. of a 1 to 10 dilution of eggwhite solution and were tested two to three weeks thereafter. Others were injected by passive transfer of a potent anti-eggwhite rabbit serum, and tested twenty-four hours later. After each animal had been killed by a blow on the head, the two uterine horns, or two apparently equal intestinal strips were suspended each in a separate bath of a Dale apparatus. This contained 150 cc. of Tyrode solution kept at a temperature of 38° C. and through which free oxygen was passed. One muscle segment served as a control, and the other was tested to the action of formaldehyde.

Experiment I. 0.5 cc. of a 1 to 10 eggwhite solution was added to each of the two baths simultaneously. The second bath had been treated previously with 0.2 cc. of neutral formalin solution (final concentration of formaldehyde about 1 to 2600). In animals that proved to be sensitive, the muscle strip contracted characteristically on the addition of egg white to the first bath, and this was recorded on a kymograph. The strip in the second bath did not contract. This bath was then emptied and eggwhite again added to fresh Tyrode's solution. No contraction occurred, and thus desensitization was simulated. On again emptying the bath, refilling with Tyrode's solution and then adding histamin, an immediate violent contraction occurred to this stimulant. This indicated that formaldehyde had not impaired the contractility of the muscle.

Experiment II. The two muscle strips were arranged as in Experiment I. To each bath egg-white was similarly added but no formaldehyde was placed in the second bath until contraction already had occurred. In about 60 per cent of the experiments formaldehyde caused relaxation of the contracted strip.

Experiments done with horse serum and horse dander extract showed similar results.

Occasionally smooth muscle was found irritable and spontaneous contractions occurred. Formaldehyde added to these strips had no appreciable effect.

From these experiments it is evident that formaldehyde may prevent and also relax smooth muscle contraction induced in anaphylaxis. Moreover, sensitized muscle exposed to formaldehyde and then failing to react to homologous antigen, will not react subsequently to such antigen, although contractility is not impaired. This effect simulated desensitization.

This is a preliminary report.

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[1] Kendall, Arthur I., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 316.

3622

Formation of Methylglyoxal From Hexose Phosphate by Tissues.

NOBORU ARIYAMA. (Introduced by P. A. Shaffer.)

From the Department of Biological Chemistry, Washington University School of Medicine.

Methyl glyoxal has long been hypothecated as a probable intermediate in the formation of lactic acid in tissues, for which hypothesis the existence of glyoxalase activity is strong but indirect evidence.

Until very recently methyl glyoxal has never been detected in tissues. Toenniessen and Fisher[1] now report that hexose phosphate with muscle in the presence of pancreas (anti-glyoxalase) forms methyl glyoxal which they were able to identify by the p-nitrophenyl-di-hydrazone. In attempting to repeat this work I find that if muscle, or, better yet, liver extract when incubated with toluol for 24 hours, then hexose phosphate added and the mixture incubated for another day, the solution contains a substance which reacts like methyl glyoxal. It may be determined by the colorimetric method, is destroyed by excess alkali as is methyl glyoxal, and it gives a red di-hydrozone with p-nitro-phenyl-hydrazine which after recrystallization from pyridin melts with decomposition at about 283° (uncorrected). The di-hydrazone made from methyl glyoxal has the same crystal form and melting point. This evidence confirms the observation of Toeniessen and Fisher and indicates that the substance is actually methyl glyoxal; but conclusion is withheld until a larger quantity of the substance is prepared and submitted for further analysis. The importance of this observation makes very desirable a detailed investigation of the reactions involved. Hexose phosphate alone, liver (or muscle) extract alone, or heated extract plus hexose phosphate does not yield the glyoxal-like substance.

The formation of methylglyoxal under the conditions stated perhaps indicates that the conversion of hexose phosphate to lactic acid takes place in steps, hexose phosphate → methyl glyoxal → lactic acid. The glyoxalase, responsible for the second step is largely destroyed during the first day of autolysis, while the ferment presumably responsible for the first step (phosphatase) is apparently still active.

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[1] Toenniessen and Fischer, *Z. f. physiol. Chem.*, 1926, clxi, 254.

3623

Protective Mechanism Against Acoustic Insult in the Mouse.

A. G. POHLMAN.

From the St. Louis University School of Medicine.

Practically all experimental animals except the small rodents show evidence of cochlear degeneration when exposed to prolonged acoustic insult. Experimental degenerations of the cochlea have been employed as a method of determining specific pitch areas in the organ of Corti. There is, however, a difference in opinion on the causal factor of the lesion. The majority hold it is due to an excessive stimulation dependent on the pitch and intensity and is, therefore, a criterion of a specific area of reception. The minority holds the degeneration does not appear until the intrinsic muscles have been fatigued and that it may represent a result of clatter in the ossicular apparatus. This would mean the lesion is not dependent on the pitch. If the mouse really escapes intact under conditions which cause lesions in other experimental animals, then an additional protective mechanism in the middle ear must be sought. Small rodents show a rather remarkable departure from the usual middle ear picture. The stapes is traversed by a large artery which must certainly act as an efficient and tireless damping mechanism.

This stapedial artery is phylogenetically responsible for the characteristic stirrup-like ossicle in the mammals as opposed to the mushroom-like columella in birds. A clatter of the ossicles in the mouse may, therefore, be automatically damped out and careful investigation is demanded, employing pure tones of known intensity. The investigation under way may throw light not only on the causal factor of the degenerations of the organ of Corti, but may also solve the problem of the reactions of the intrinsic muscle as a damping mechanism to eliminate clatter, as well as to adjust the ossicles to the variable topography of the middle ear.

3624

Steam Distillation of Volatile Fatty Acids From a Saturated Salt Solution.

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From the Department of Internal Medicine, Washington University School of Medicine.

During a study of the volatile fatty acids of stools, a method was sought for their rapid distillation. Duclaux[1] showed that each volatile fatty acid has a specific distillation rate. Fractional distillates collected from mixtures of these acids contained the acids in proportion to their concentration and their respective distillation rates. Direct distillation for the identification of volatile fatty acids is inaccurate. Dyers method of steam distillation provides a means for fairly accurate determination of the mixture of two or three acids.

Our problem was the distillation of fatty acids from filtrates of stools; the organic matter having been precipitated. The filtrates contained large amounts of inorganic salts which could be expected to disturb the distillation rates even when obtained by steam. Some advantage would be obtained by accelerating the rates of distillation of such acids as formic and acetic. Also added accuracy would be obtained in the identification of these acids if the differences in their distillation rates could be increased.

It has long been known that proprionic and butyric acids may be salted out of solution by adding sodium or calcium chloride to saturation. It was found that if steam is passed through a saturated salt solution containing a volatile acid, the rate of distillation of the acid is greatly accelerated. The method of procedure is as follows:

To 100 cc. of water containing from 5 to 20 cc. of a 0.1 N solution of formic, acetic or butyric acid, 70 grams of magnesium sulphate was added, 2 cc. of 50 per cent solution of sulphuric acid, to assure strong acidity. Steam was passed through the salt mixture at such a rate that ten to fifteen minutes were required to collect each 100 cc. fraction of distillate. Six 100 cc. samples of distillate were collected. The volume of the salt solution was kept rigidly constant. Phenolphthalein was used as an indicator in titrating distillates. The rates of distillation of formic, acetic and butyric acids are as follows:

TABLE I. *Distillation of Volatile Fatty Acids.*

	100 cc.	200 cc.	300 cc.	600 cc.
	Per cent.	Per cent.	Per cent.	Per cent.
Formic	29	52	66	90
Acetic	63	86	93	98
Butyric	92	96	97	98

Mixtures of these acids when distilled under the above conditions showed rates in proportion to their concentration and their specific rates of distillation.

The advantages of the method are, that the acids are more rapidly distilled; that the solutions of acids contaminated by inorganic salts may be distilled, and that the difference in rate of distillation between formic and acetic acids is greatly increased.

This is a preliminary report.

3625

Effect of Cyclic Changes in Female Guinea Pig on Cell Proliferation in Epidermis.

LEO LOEB AND F. L. HAVEN.

From the Department of Pathology, Washington University School of Medicine.

It occurred to us that the epidermis may be a tissue in which cell proliferation can be readily determined in a quantitative manner and that in this way a method might be worked out which would allow us to determine the effect of various conditions and substances on cell growth in general. We have therefore determined quantitatively the number of mitoses occurring in the epidermis of several species of animals and also the number of cells in the lower and upper layers of the squamous epithelium.

In this report we shall limit ourselves to a statement concerning the differences which we found in the mitotic proliferation of the epidermis of the guinea-pig in normal adult males on the one hand, and in females in various stages of sexual activity, on the other hand. We found that in adult female guinea pigs during the sexual cycle the mitotic cell proliferation is distinctly less active than in adult males otherwise living under the same conditions. It seems to be weakest at the time of oestrous and then rises very slowly towards the end of the cycle, without, however, reaching the average of the mitotic activity in the male even as late as 15 to 16 days following heat. In castrated female guinea pigs cell proliferation is low; we must assume that castration as such lowers the proliferative activity of the epidermis. On the other hand in pregnant guinea pigs it is definitely higher than in animals during the ordinary cycle, although it does not reach the average of adult male guinea pigs. This rise which takes place during pregnancy, to some extent is maintained during the period of nursing, although it is then not quite so high as during the former period.

We see thus that the changes taking place in female guinea pigs during the sexual cycle and even in pregnancy have a depressing effect on the proliferative activity of the epidermis. This effect is not caused by the sex constitution as such; this is shown by the fact that young, sexually immature female guinea pigs show at least as great a proliferative activity as males of similar age and perhaps an even greater activity.

This is a preliminary report.

3626

Notes on the Development of Lymphocytes.

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From the Research Laboratories of the Barnard Free Skin and Cancer Hospital, and the Department of Surgery, Washington University School of Medicine.

The author has noticed that fully matured lymphocytes, polymorphonuclear leucocytes and many other mononuclear cells will not grow in a tissue culture. Other reactions of these cells when compared with those of fixed tissues indicate that they have suffered decided changes from the mother cells from which they arise.

The migration of fixed tissue cells, including the mother cells of the lymphocytes and leucocytes, is the result of their liberating a lipoid blood coagulating substance which has been named the ergusia. Their growth is also dependent upon this same substance. The migration of the leucocytes and lymphocytes is not associated with the liberation of this coagulating substance. They migrate apparently by dissolving paths in a solid clotted medium. They possess fermentative reactions, but have lost their power to liberate the ergusia in their differentiations.[1]

They are not important in aiding the body against invading organisms. Bacteria placed in the medium near a fragment of spleen causes this fragment to be disintegrated almost entirely into lymphocytic cells.[2] These lymphocytes show, however, no resistance to the bacteria. They migrate readily and in practically every case directly in and among the growing bacterial cells. Fixed tissue cells on the other hand are strongly antagonistic to these growing bacteria.[1] The fixed tissue cells invade areas of the medium containing bacteria only when their growth activity is greater than that of the bacteria. The bacteria then cease to grow in their presence. This is strikingly seen in cultures of actively growing embryonic tissue or of cancer.[3] When the bacteria are growing very actively the fixed tissue cells cease to become active at the boundary of the zone of activity of the bacteria.[4]

These facts led to the question whether the lymphocyte is any more than the "ashes" of some much more general fundamental reaction in the organism. It acts more as a degenerate cell than one which takes an active part in body defense. It became of interest to study the conditions of the differentiation or formation of these cells.

Several years ago the author placed in tissue cultures fragments of the spleen of a one-week old kitten in drops of the kitten's own plasma, and in drops of plasma prepared from the blood of the mother. The reaction of the splenic cells in these two cases was strikingly different. The spleens of these young kittens show very little differentiation of lymphocytes. In the mother's plasma large numbers of lymphocytes migrated from fragments of them into the medium. In the kitten's plasma no lymphocytes appeared, but there was a most active growth of mesenchyme-like cells. Several repetitions of these experiments have always given the same results.

Last summer Mr. Kramer and I noted in a few experiments that this ability for the plasma of animals to produce lymphocytes from the spleen of young animals appears in kittens to a certain extent as early as two or three weeks after birth.[5]

In other experiments Jorstad and the author[6] noted that vitamin A is probably only the ergusia of plants and animals used for food. The question arose, therefore, can the differentiation of these cells be in any way associated with the loss or the formation of this vitamin in the tissues. Children dying from vitamin A deficiency often show a marked hyperplasia of the lymphoid tissue. Murphy[7] notes an accumulation of lymphoid cells in areas treated with small doses of x-rays or an increased resistance of these areas to cancer. We note that small doses of x-rays protect animals for a time against a vitamin B deficiency, while slightly larger doses protect them also for a time against a vitamin A deficiency. In experiments with growing embryomas of mice, one persisted and grew throughout the life of the mouse.[9] Mouse embryos generally grow for a very short time and are then absorbed. The persisting embryoma showed a marked infiltration with lymphocytes. I have shown that the growth of these embryomas is associated with a low value of the ergusia and the growing cells use ergusia for their growth.

It thus became of interest to study these lymphoid reactions in animals suffering from changes in their vitamin content. Mr. Paul Guttman has studied, therefore, the growth of embryomas of rats on a dietary deficient in vitamin A. In his first experiments he found the embryonic tissue will not grow in animals which were placed immediately on the deficient diet. In later experiments he then placed the animals on this diet after the embryomas had begun to grow. In each case he found a marked infiltration of lymphocytes in these tumors. The lymphoid infiltration in these cases was associated also with hyaline and degenerative changes in the embryonic tissue.

If these embryomas had not degenerated to too great an extent they grow rapidly again when the host is placed on a good dietary. While we do not know the eventual outcome of such embryomas, there has been no evidence to show that the lymphoid cells contained in them prevent in any way their subsequent growth.

In the tissue culture the growth of all cells is dependent upon certain definite supply of lipoid substance, the ergusia. It is only when this lipoid is present in excess in and about the cells that it inhibits their growth.[9, 10, 11] Actively growing tissues remove this lipoid from differentiated tissues about them, producing hyaline and degenerative changes.[10, 12] Hyaline and degenerative changes are associated with the infiltration of lymphocytes in the tissues when the vitamin A is removed from the diet of the hosts.

There has been no evidence from these studies, therefore, to show that the lymphocytes act to resist the invasion of cells. They are evidently only one of the results of a decrease in those lipoid elements of the tissues which are necessary for the growth of the invading cells.

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[1] Burrows, M. T., *Am. J. Anat.*, 1926, xxxvi, 289.

[2] Burrows, M. T., unpublished notes.

[3] Burrows, M. T., Burns, J. Edw., and Suzuki, Y., *J. Exp. Urol.*, 1917, i, 3, Fig. 4.

[4] Burrows, M. T., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 495.

[5] Burrows, M. T., unpublished notes.

[6] Burrows, M. T., and Jorstad, L. H., *Am. J. Physiol.*, 1926, lxxvii, 38.

[7] Murphy, J. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[8] Burrows, M. T., Jorstad, L. H., and Ernst, E. C., *J. Am. Med. Assn.*, 1926.

[9] Burrows, M. T., *J. Can. Res.*, 1925, ix, 224.

[10] Burrows, M. T., to appear in the *Arch. Path. and Lab. Med.*

[11] Burrows, M. T., *J. Can. Res.*, 1926, x, 239.

[12] Burrows, M. T., to appear in the *J. Can. Res.*

3627

Studies on the Mechanism of Gastric Hydrochloric Acid Secretion.

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The following study was planned to throw some light upon the mechanism by which hydrochloric acid is secreted by the stomach. The serum electrolytes of the arterial and of the venous bloods from the stomach were determined before and during gastric secretion in dogs under amytal anesthesia, histamine being used to stimulate secretion. Venous blood from the acid producing portion of the organ was obtained from the coronary vein. The total base was determined by a modification of Fisk's urine method. The anions are expressed in millimols of base combining capacity, assuming a ratio of primary to secondary phosphate of 1 to 4, and calculating the base combined with protein by a formula determined by Van Slyke. The difference between the total determined acids and the total base is taken as representing the organic acid fraction though it contained the small amount of sulfate which was not estimated.

In studies in this laboratory on human subjects it has been shown that during hydrochloric acid secretion the chloride concentration of the gastric juice continues, as in the fasting condition, to be about the same order of magnitude as in serum, but that there is a decrease in base proportional to the increase in free hydrochloric acid. This is similar to the observations of Gamble and McIver[1] on dogs. If chloride ions and water are thus secreted at about the same rate little change in the concentration of chloride in the serum from the secreting stomach would be expected. The accompanying table shows clearly that this assumption is correct; there is never more than a slight fall in chloride. But, as water and chloride ions are secreted there is a retention of base, thus liberating free hydrochloric acid. Some factors must therefore be operative to compensate for this increase in base and replace any slight decrease in chloride. The data indicate that this adjustment is partially effected by a gain in bicarbonate. But it is evident that the bicarbonate supplied fails to entirely accommodate for the changes. Organic acids appear to play an equally prominent part. In fact, in the experiments on Dog V the gain in bicarbonate was relatively unimportant, while there was a striking increase in the organic acid fraction. It seemed possible that phosphate might be significant in this replacement mechanism, but any direct action appears to be trivial. No reaction is suggested whereby the weak acids, carbonic and possibly lactic, usual products of cell activity, could replace the strong hydrochloric acid.

TABLE I.

Changes in serum electrolytes of arterial and venous bloods from the stomach before and during gastric secretion, in one of the dogs.

DOG V.

	Before Secretion			After ¾ hr.		After 1¼ hour			After 2¼ h.	
	Arterial	Arterial	Venous from stomach	Arterial	Venous from stomach	Arterial	Venous from stomach	Venous from leg	Arterial	Venous from stomach
	mM	mM	mM	mM	mM	mM	mM	mM	mM	mM
HCO_3	23.8	23.5	23.3	21.5	24.9	21.3	24.9	23.9	24.0	25.6
Cl	113.0	112.2	111.8	111.8	111.9	110.8	109.0	109.9	110.2	108.1
PO_4	3.1	3.0	3.0	4.1	4.1	4.1	3.7	4.1	4.2	5.4
Protein	10.7	11.5	11.5	10.0	9.8	10.4	11.4	11.9	10.6	9.1
Total acid	150.6	150.2	149.6	147.4	150.7	146.6	149.0	148.9	149.0	148.2
Total base	175.0	175.0	171.5	189.8	199.8	185.8	193.0	184.3	186.4	197.7
Organic acid	24.4	24.8	21.9	42.4	49.1	39.2	44.0	35.4	37.4	49.5

This is a preliminary report.

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[1] Gamble, J. L., and McIver, M. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 439.

Minnesota Branch.

University of Minnesota, Minneapolis, Minn., May 25, 1927.

3628

Observations on the Function of the Pylorus as Revealed by Duodenal Regurgitation.

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The recent attention given to Boldyreff's theory[1, 2, 3] of the automatic regulation of the acidity of the stomach through duodenal regurgitation, has opened up an important line of clinical investigation. Two methods of determining regurgitation are being employed, the presence of bile in the stomach, and the estimation of the total chlorides of the stomach content as compared with the free or total acid.

We have investigated the presence of trypsin and bile in the fasting stomach, as well as their disappearance and reappearance in the stomach in the presence of water and dilute acid and alkali.

Nine examinations were made on cases as follows:

5 total achylia, giving no acid on fractional meals or after injection of histamine (pernicious anemia).

1 normal.

1 low duodenal obstruction from retroperitoneal growth.

1 duodenal ulcer.

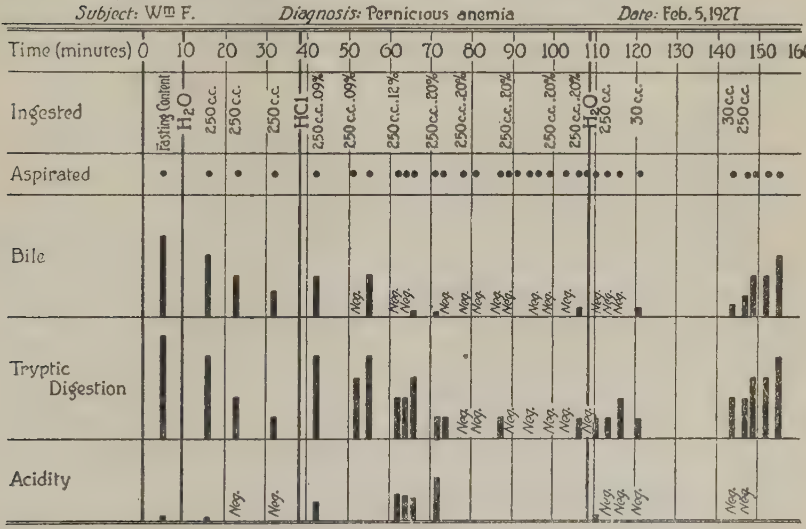
1 pyloric resection by the Polya method.

In carrying out the experiments, the contents of the fasting stomach were removed through a Rehfuss tube with the olive located in the lower part of the stomach. The stomach was then washed at 3 to 5 minute intervals with small amounts of water or of the reagent selected, and samples immediately withdrawn; or the reagent was allowed to remain in the stomach and portions fractioned out at 5 to 10 minute intervals.

In testing for the presence of trypsin, the stomach content was centrifuged or filtered if opaque, the clear fluid was then shaken with permutit to remove ammonia and a solution of purified egg albumin added to each. The mixture was then brought to a pH of 8.5 (just alkaline to phenolphthalein) and allowed to incubate 48 hours at 37°, when amino acids were tested for by Folin's method.

In cases of achylia we found trypsin almost continually present when the stomach was washed with water, 1 per cent Na_2CO_3 or HCl (less than 0.05*N*). With more concentrated acid trypsin usually disappeared. The presence of bile was more variable. Though this was a constant finding in achylia, in other types of cases less uniformity of result was obtained.

Shows the presence in the stomach of bile and trypsin during washings with water and dilute acid, their complete disappearance during washings with more concentrated acid



This shows a typical experiment in a case of achylia. Bile and trypsin were both present during washing with water and 0.09 per cent HCl. During washing with 0.2 per cent HCl it disappeared completely and reappeared only intermittently and in minute amounts. Water was then introduced, whereupon trypsin reappeared at once. Later on, bile also reappeared.

From this, as well as others of our experiments it was shown definitely that bile and trypsin appear independently of each other, trypsin occurring with greater frequency. Bile only rarely appeared in the absence of trypsin. This point we consider of importance, since Baird, Campbell and Hern,[4] in discussing the neutralizing effect in the stomach of the pancreatic juice have concluded the absence of pancreatic juice in the stomach from the absence of bile. The anatomic explanation of our findings is suggested by a study of the anatomy of the pancreatic ducts by Baldwin,[5] showing a patent and probably functioning accessory duct in 77 per cent of cases studied and a separate opening of the pancreatic and bile ducts in 25 per cent of the cases.

Whether the disappearance of trypsin and bile after washing the stomach with acid is due to the closure of the pylorus or to a closing effect of the separate ducts from the liver and the pancreas we are not prepared to state. We wish to emphasize that wherever the closing mechanism is located, the effect in the pernicious anemia cases is a prolonged closure, lasting from 30 to 40 minutes. In a case of healed duodenal ulcer the closing effect was much more marked, more than an hour and a half elapsing before signs of duodenal regurgitation occurred after the introduction of acid into the stomach.

This is a preliminary report.

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[1] Boldyreff, W., *Zntrlblt. f. inn. Med.*, 1908, vi.

[2] Boldyreff, W., *Ergebnesse d. Physiol.*, 1911, xi, 121.

[3] Boldyreff, W., *Quart. J. Exp. Physiol.*, 1915, viii, 1.

[4] Baird, M. McC., Campbell, J. M. H., and Hern, J. R. B., *Guy's Hosp. Rep.*, 1924, lxxiv, 23.

[5] Baldwin, W. M., *Anat. Rec.*, 1911, v, No. 5.

3629

The Prenatal Growth of the Human Thymus.

RICHARD E. SCAMMON.

From the Department of Anatomy, University of Minnesota.

The weight of the human thymus in the fetal period is characterized by great variability. Therefore a large number of observations are necessary to demonstrate even the approximate course of prenatal growth of this organ.

The ponderal growth of the thymus, with respect to body-weight, has been studied in a series of 1043 weighings of the organ from human fetuses under 4000 grams in total (dead) body weight. In no instances were observations made on fetuses living over 48 hours.

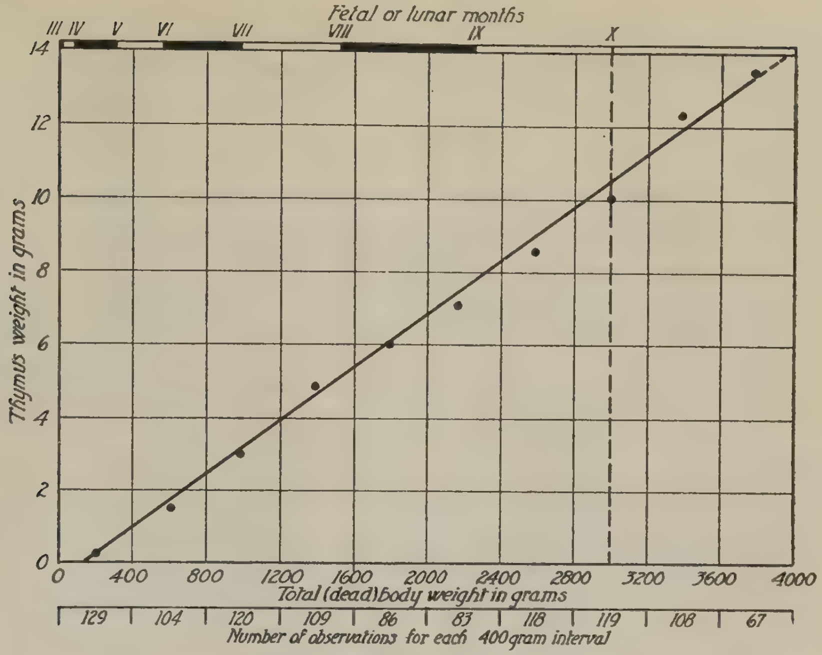

FIG. 1.

Illustrating ponderal growth of the thymus with respect to the total body-weight in the fetal period. The body-weight (in gm.) is base line of the graph. Estimated age[1] is represented by panels, upper margin of figure. Observed, mean, thymus-weights for 400 gm. intervals of total body-weight are indicated by dots. Graphical expression of empirical formula for relation of thymus-weight to body-weight is heavy solid line. Number of observations in each 400 gm. interval of body-weight is indicated in lower panel of graph. The vertical broken line in the field represents computed birth value of body-weight.

As shown in Figure 1, the relation appears to be rectilinear. It may be approximated by the empirical formula:

$$\mathrm{TW} = 0.0036418\ \mathrm{BW} - 0.474 \quad (1)$$

where "TW" is the weight of the thymus in grams and "BW" is the (dead) weight of the total body in grams. This formula was computed from the means of thymus-weight and of body-weight for the ten 400 gram intervals of body-weight from 0 to 4000 grams (weighting by the square root of the number of observations in each interval). The calculated values show a mean, weighted, absolute deviation of 0.248 grams from the observed averages. The mean, weighted, relative deviation is 6.03 per cent. This relative deviation lies mainly in the two lower weight-intervals, the mean, weighted, relative deviation for the upper eight intervals being 3.31 per cent.

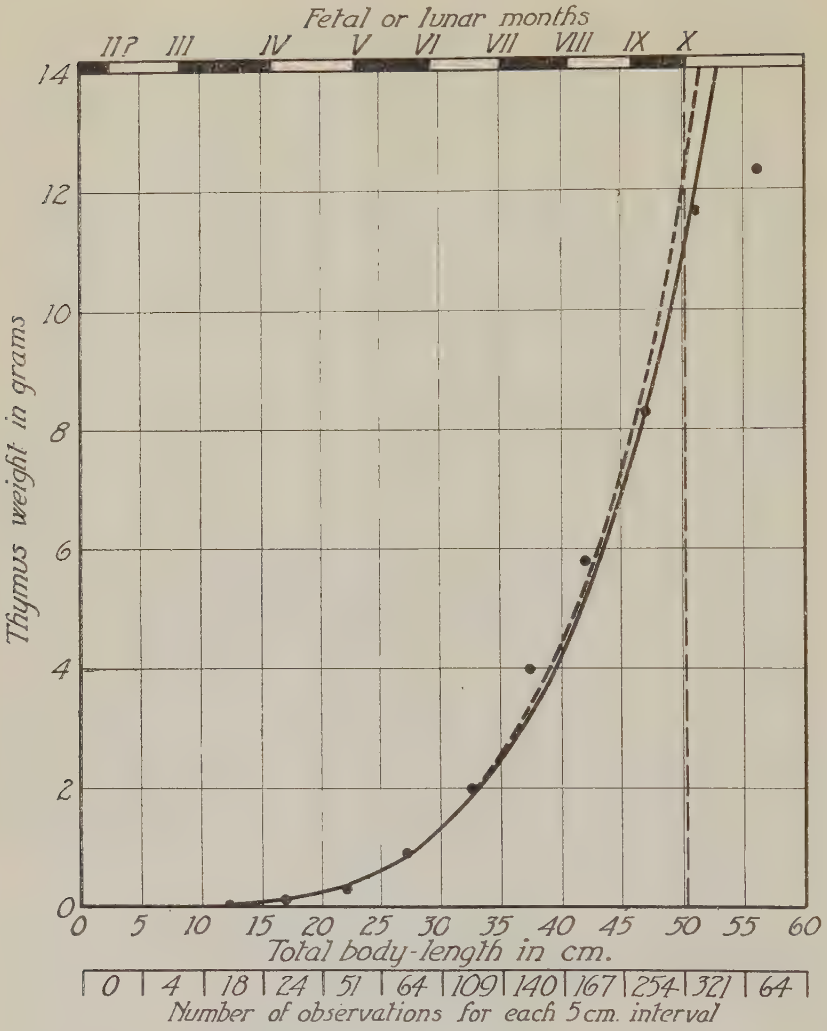

FIG. 2.

Illustrating ponderal growth of the thymus with respect to crown-heel body-length. The body-length is on base line of graph. Estimated age[2] is represented along the upper margin of the figure. The observed, mean, thymus-weights for 5 cm. intervals of total body-length are indicated by dots. Graphical expression of the first empirical formula for the relation of thymus-weight to body-length is represented by the solid curve. The graphical expression of the second empirical formula is represented by the heavy broken curve. The number of observations in each 5 cm. interval of body-length is indicated in the lower panel of the graph. The vertical broken line in the field represents the computed birth value of body-length.

The relation of thymus-weight to body-length is shown in Fig. 2. As in the preceding series, in no instances were observations made on fetuses living over 48 hours. Two approximate, empirical expressions have been developed for this relationship, the first by using the mean thymus-weights and body-lengths for the 5 centimeter intervals of body-length from 5 to 60 centimeters (based upon 1216 observations), and the second by using the means of thymus-weight and body-length for the 5 centimeter intervals from 5 to 55 centimeters (based upon 1152 observations). In each case the expression was calculated by the method of means, weighting by the square root of the number of cases in each 5 centimeter interval of body-length.

The expression obtained by the first procedure is:

$$TW = 0.0001312\ L^{4.0654} \quad (2)$$

where "TW" is the thymus weight in grams and "L" is the total or crown-heel length of the body in centimeters. The calculated values obtained by this expression show a mean, weighted, absolute deviation of 0.700 grams from the corresponding observed averages. The mean, weighted, relative deviation is 13.52 per cent.

The expression obtained by the second procedure is:

$$TW = 0.0001076\ L^{4.129} \quad (3)$$

The calculated values of this expression show a mean, weighted absolute deviation of 0.511 grams from the corresponding observed averages and the mean, weighted, relative deviation is 12.286 per cent. Neither of these formulae can be regarded as entirely satisfactory expressions for the relation between thymus-weight and stature.

The formulae indicate a closer correlation between thymus-weight and body-weight than between thymus-weight and body-length. The constants of the empirical formula for thymus-weight and body-weight indicate an increase in relative weight of the thymus in the fetal period, a phenomenon previously noted by Legou,[3] Jackson[4] and others. This report is preliminary to a more detailed biometric study of the growth and variability, and possible sex differences, of the thymus in prenatal life.

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[1] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 355, (formula 1).

[2] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 157 (formula 5).

[3] Legou, E., *Quelques considérations sur le développement du foetus,* Thesis, Paris, 1903.

[4] Jackson, C. M., *Am. J. Anat.*, 1909, ix, 117.

3630

A Quantitative Study of the Pettenkofer Reaction for Bile Salts.

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From the Laboratory of Physiological Chemistry, University of Minnesota.

As originally described this is a qualitative test for bile salts and is performed by dissolving a small amount of bile, or, the bile containing fluid, in concentrated H_2SO_4 and adding a few drops of a 10 per cent solution of cane sugar. In the presence of bile salts the solution assumes a red-purple color which approaches a violet within the course of a day. As shown by a number of investigators, the reaction may be satisfactorily carried out in a 50 per cent H_2SO_4 solution.

In attempting to use the Mylius modification of the Pettenkofer reaction quantitatively, which employs furfural instead of cane sugar, it was never possible to obtain color matches. It has been found that by performing the reaction in weaker concentrations of H_2SO_4 (30 to 40 per cent) a blue color is developed. This color is due to an absorption bond at 6200 A and seems suitable for quantitative colorimetric determinations. Color matches are easily obtained when the solution being tested contains twice as much bile salt as the standard. When the tested solution is within 50 per cent of the strength of the standard the error is never greater than 5 per cent. It is usually about 2 per cent. This error increases to 10 per cent when the tested solution differs from the standard by 100 per cent.

Glycocoll, taurine, cholesterol, a cholesterol ester (lanolin), lecithin, kephalin, and oleic acid do not give any color reaction in the weaker H_2SO_4 solution mentioned. Oleic acid and the phosphatides are known to give a red color when the reaction is carried out in higher concentrations of H_2SO_4. Isobutyl and amyl alcohol also give a red color reaction with furfural in 50 per cent H_2SO_4. The color produced by the alcohols in the strength of H_2SO_4 we use is likewise red. The blue color produced as described seems to be more specific for the bile salts than does the red-purple color. The blue color also seems to be much more suitable for quantitative colorimetric determinations.

This is a preliminary report.

3631

Bacteriophages in Bacterial Cultures.

E. T. DEWEY AND R. G. GREEN.

From the Department of Bacteriology and Immunology, University of Minnesota.

In carrying out examinations of fecal specimens for the presence of bacteriophage, the 11 strains of *Eberthella typhi* from the American Type Culture Museum were utilized, together with 14 cultures of other organisms from our own laboratory. It was noted that most of the strains of *Eberthella typhi* were sensitive to bacteriophage action, but that two cultures appeared to be very resistant. The relative susceptibility of these cultures is shown by results from 39 fecal samples which contained bacteriophage active against one or more typhoid strains. The findings of the third generation against the 11 typhoid strains and 2 strains of *Escherichia coli* are shown in Table I.

TABLE I.
Relative Susceptibility to Bacteriophage Action.
39 fecal specimens. 3rd generation.

No.	Strain	Source	No. times susceptible
1	168	A. T. C. Museum	38
2	169	”	38
3	191	”	6
4	192	”	27
5	193	”	28
6	194	”	25
7	195	”	24
8	196	”	0
9	197	”	28
10	564	”	32
11	Thompson	”	22
12	B 70 A	Minn. Museum	6
13	B 71 A	”	0

Of the *Eb. typhi* cultures it is seen that strains 191 and 196 were very resistant to bacteriophage action. As no lysis of *Es. coli* B71A occurred while B70A was lysed 6 times, it would appear that B71A was relatively resistant.

The resistance of these cultures might be due to the presence of a bacteriophage in the cultures. In testing for the presence of a bacteriophage 12 more cultures were added to the group, making a total of 25. The additional cultures were: 3 species of *salmonella,* 1 strain *Eb. dysenteriae,* 1 strain *Eb. paradysenteriae,* 1 strain *Proteus vulgaris,* 3 species of *staphylococcus,* 2 species of *Aerobacter.*

These organisms were inoculated into plain broth and incubated for 24 hours. At the end of that time each culture was filtered through separate Berkfelt candles and the filtrates of each tested for activity against all 25 organisms. The test was performed by adding to a tube of plain broth 3/10 cc. of a 6 hour growth of the organism, and adding immediately following, 3/10 cc. of the filtrate to be tested. The culture was incubated 24 hours and read by comparing with a 24 hour growth used as a control. This reading represented the 1st generation. The broth was then filtered and the filtrate tested in an identical manner, the 2nd reading being the 2nd generation.

Of the 25 filtrates tested, filtrates from cultures 191, 196, and B71A were the only ones showing bacteriophage activity and their action was confined to the organisms listed in Table I. The activity of these filtrates in the 1st and 2nd generation is shown in Table II. In representing the comparative activity of the filtrates, the following definitions are used:

4+ Maximum bacteriophage action, no demonstrable growth, tube as clear as plain broth.

3+ Marked bacteriophage action, medium appears clear, growth apparent only by agitation.

2+ Definite bacteriophage action, marked clearing compared with control.

1+ Indefinite bacteriophage action, definite inhibition of growth when compared with control.

— No bacteriophage activity evident. Tube about as turbid as control.

TABLE II.

Bacteriophage Activity of Culture Filtrates.

			Cultures of Bacteria.													
		Gen.	168	169	191	192	193	194	195	196	197	564	Thompson	564	B70A	B71A
Culture Filtrates	191	1st	3+	3+	—	2+	—	2+	2+	—	—	2+	2+	—	—	—
		2nd	3+	3+	—	2+	—	—	2+	—	—	2+	—	—	—	—
	196	1st	3+	2+	—	2+	—	—	2+	—	—	1+	3+	—	—	—
		2nd	4+	3+	—	2+	—	—	2+	—	—	1+	3+	—	—	—
	B71A	1st	—	—	—	—	—	—	—	—	—	—	—	—	2+	—
		2nd	—	—	—	—	—	—	—	—	—	—	—	—	2+	—

The culture filtrates in each case were inactive against the culture from which they were derived. The active filtrates were most active against cultures 168 and 169 which were also the most susceptible to the filtrates from fecal specimens.

The American Type Culture Museum's *Eb. typhi* cultures Nos. 191 and 196 apparently carry with them a bacteriophage. These bacteriophages are almost identical in their activity on the other museum strains. The resistance of these cultures to the bacteriophage action of feces filtrates is explained by the presence in the cultures of a bacteriophage. The cultures consist almost entirely of resistant cells, but there is probably produced in the growing culture enough susceptible cells to allow the bacteriophage to continue to multiply and maintain its presence.

Conclusion. 1. Cultures No. 168 and No. 169 are the most susceptible to bacteriophage action and are the most dependable to demonstrate typhoid bacteriophages in feces filtrates.

2. Cultures No. 191 and No. 196 are very resistant to bacteriophage action and give little indication of the presence of a bacteriophage when used as test organisms.

3. Cultures No. 191 and No. 196 carry with them a bacteriophage exhibiting activity against most other strains, but showing no reciprocal or homologous activity.

3632

Biological Study of Mushroom Extract and Effect of Sodium Ricinoleate on its Toxicity.

R. G. GREEN AND A. V. STOESSER.

From the Department of Bacteriology and Immunology, University of Minnesota.

A watery extract of the poisonous mushroom, *Amanita-phalloides*, was prepared according to the method of Ford.[1, 2] The extract was concentrated and sterilized by filtration. Using 1 cc. of a 5 per cent suspension of red blood corpuscles, 0.02 cc. of the extract produced complete hemolysis of guinea pig corpuscles. When injected into guinea pigs 1 cc. of the extract was sufficient to kill a 300 gm. guinea pig in 5 days. Upon the addition of sodium ricinoleate a precipitate was formed. A modified extract was prepared by the addition of sodium phosphate (Na_3PO_4), the precipitate being removed by filtration. Modified extract gave a clear, dark brown solution with sodium ricinoleate. 0.10 of this extract was necessary to hemolyze 1 cc. of a 5 per cent suspension of guinea pig erythrocytes. 1 cc. of the modified extract killed a 300 gm. guinea pig in 7 days. The addition of non-toxic amounts of castor oil soap to the mushroom poison greatly increased its toxicity. The addition of 0.03 gm. of castor oil soap to 1 cc. of toxin reduced the life interval from 7 days to 1½ hours. A similar increase in toxicity has previously been noted by Larson[3] upon the addition of castor oil soap to Botulinus toxin. Various mixtures of castor oil soap and mushroom toxin were studied and it was found that increase in toxicity depended both upon the concentration of the soap and the concentration of toxin.

This is a preliminary report.

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[1] Ford, *J. Infec. Dis.*, 1906, iii, 192.

[2] Ford, *J. Exp. Med.*, 1906, viii, 437.

[3] Larson, *Ibid.*, 1924, 278.

3633

The Increase in Toxicity of Mushroom Poison Produced by Sodium Ricinoleate.

R. G. GREEN AND A. V. STOESSER.

From the Department of Bacteriology and Immunology, University of Minnesota.

It has been found by the authors that mixtures of sodium ricinoleate and mushroom poison were more toxic than the mushroom poison alone. Experiments were carried out upon the effects produced by the separate injection of the two components into guinea pigs. Simultaneous but separate injections of mushroom toxin and castor oil soap gave toxic effects comparable to the injection of a mixture. The toxic effect was also evident if the injection of either component was delayed up to 48 hours. After the injection of an M.L.D of toxin, 1 cc. of soap injected 48 hours later, reduced the life interval from 7 days to 1 day. After the injection of 1 cc. of a 3 per cent soap solution, an M. L. D. of toxin injected after 48 hours produced a fatal termination in 2 days instead of 7 days. Various combinations of dosage at different intervals between injections showed that the longer the interval between injections, the larger must be the dose of toxin to produce corresponding results. With the injection of 0.03 gm. of soap, death is produced by the simultaneous injection of 0.1 M. L. D. of toxin. If, however, the injection of 0.1 M. L. D. is delayed 3 hours, the toxicity is not developed.

However, at this time 1/5 M. L. D. will cause a fatal toxemia. These results indicate that the binding or elimination of the injected castor oil soap is appreciable inside of the first 3 hours. The delayed injection of one M. L. D. of toxin shows that soap is still present in the animal body 72 hours after injection.

This is a preliminary report.

3634

Effects of Water Content on Muscular Efficiency.

F. R. STEGGERDA. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

Since little or no specific knowledge is to be had regarding water and the physiological activity of cells, it was thought desirable to investigate the relationship between the variation in total water content and muscular efficiency.

The gastrocnemii muscles of normal frogs were removed by the usual method. After weighing they were suspended in an ordinary muscle chamber. Contractions were obtained by stimulating with an electric current of known strength. All the muscles were forced to lift a known weight, thus leaving the variation in height of contractions as the criterion of efficiency. Readings were made at one-half to one-hour intervals, with the experiments extending from 3 to 6 hours.

The variation in water content was produced osmotically by using Ringer's solution of different concentrations. All the solutions were prepared from a known stock solution. The muscles were immersed in the solutions at all times except when contractions were being taken.

The right gastrocnemius in all cases served as a control and was submerged in normal Ringer's fluid, whereas the left gastrocnemius of the same frog served as the experimental muscle and was submerged in either hypotonic or hypertonic Ringer's solution of known concentrations. After the experiment was completed, the muscles were again re-weighed and the percentage variation in weight was determined. The degree of weight change was dependent upon the strength used and presumably is due to alteration of the water content of the cells.

Fifty-two different experiments were carried on using various strengths of Ringer's solutions. After taking the average for the different groups of experiments, curves were plotted, showing the relationship between the weight alteration and the changes in muscular efficiency. It was found that muscle tissue was able to increase between seven and eight per cent in weight before efficiency fell below that of normal, whereas if a decrease in weight was present, efficiency was noted to fall below normal almost immediately.

This is a preliminary report.

3635

Effect of Unilateral Nephrectomy Upon Number of Renal Corpuscles in Young Albino Rats.

MARGARET A. SHIELS. (Introduced by C. M. Jackson.)

From the Department of Anatomy, University of Minnesota, Minneapolis.

When one kidney is absent, the other undergoes the well-known compensatory hypertrophy. Most investigators agree that in cases of congenital defect the renal corpuscles (glomeruli) of the enlarged kidney appear to be increased in number (hyperplasia). In cases of postnatal, acquired renal defect, however, at least in adults, the renal corpuscles appear to be merely increased in size (hypertrophy) without increase in number. Even in young animals, the recent investigations of Arataki[1] (albino rat at 20 days) indicate no increase in the number of renal corpuscles after unilateral nephrectomy.

But these experiments were performed upon animals in which the normal formation of new renal corpuscles had almost or entirely ceased. Kittelson[2] has shown that in the rat the corpuscles have nearly reached the adult maximum number at three weeks of age. It seems possible that the removal of one kidney in the rat shortly after birth, when the new formation of corpuscles is still active, might produce a corpuscular hyperplasia, comparable to that found in cases of congenital absence of one kidney.

In order to test this possibility, the present study was undertaken. In a female albino rat one week old, body weight 9.09 gm., the right kidney was removed. It weighed 0.049 gm. Two weeks later the rat was killed at 21 days of age, body weight 24.4 gm. The left kidney weighed 0.214 gm., which is about 34 per cent above Donaldson's[3] norm for kidney weight (female) at corresponding body weight.

Both kidneys were cut in serial sections and the total number of renal corpuscles in each kidney was counted by Kittelson's method. In the right kidney, originally removed by operation, 8453 fully formed corpuscles were counted. There were also many (uncounted) corpuscles observed in various stages of formation in the peripheral, nephrogenic zone of the renal cortex. In the hypertrophied left kidney, removed later at autopsy, 26,779 corpuscles were counted. This is but slightly above the number in the normal rat of this age and body weight as found by Kittelson (25,930) and by Arataki (female, 24,435).

This difference is so small that it probably represents insignificant individual variation, rather than an actual increase in the number of corpuscles. We may, therefore, conclude that there is apparently no hyperplasia of the renal corpuscles in the albino rat, even when unilateral nephrectomy is performed at a very early age, during the period when the new formation of corpuscles is still actively in progress.

Whatever may be the factor or factors which determine the number of renal corpuscles (and tubules) formed in the nephrogenic blastema, it is evident that this phase of the developmental process is not influenced by the functional changes resulting from unilateral nephrectomy.

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[1] Arataki, M., *Am. J. Anat.*, 1926, xxxvi, 437.

[2] Kittelson, J. A., *Anat. Rec.*, 1917, xiii, 385.

[3] Donaldson, H. H. The rat. Reference tables and data. 2nd ed. Wistar Institute of Anatomy. Phila. 1924.

3636

Study of Reversible Oxidation of Adrenaline and Its Derivatives.

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Ephedrine, adrenalone, methylacetopyrocatechol, dimethylacetopyrocatechol, adrenaline, the ethyl and methyl ethers and the anhydride of adrenaline were investigated to see whether (a) they would be oxidized by dibromophenolindophenol, naphtholidichloroindophenol, methylene blue and indigo carmine; (b) whether the oxidized forms would act as oxidizing agents with reduced indigo; (c) whether they formed reversible oxidation-reduction systems; (d) whether under any conditions they would function as cyclic catalysts.

All of the eight substances, except ephedrine, are oxidized by dibromophenolindophenol. Adrenaline and its ether derivatives are oxidized by only the last mentioned oxidizing dye. Adrenalone, methylacteopyrocatechol, and dimethylacetopyrocatechol are oxidized by naphtholdichloroindophenol and indigo carmine. Adrenalone and methylaminoacetopyrocatechol are oxidized by all of the dyes. Dimethylacetopyrocatechol is not oxidized by methylene blue, but is oxidized by indigo carmine. These results emphasize the fact that the configuration of the oxidizing dyes is of greater importance than the intensity of oxidation produced by the dye.

Adrenalone, methyl and dimethylacetopyrocatechol will oxidize reduced indigo after they have been oxidized with the dyes. The oxidation is quantitatively reversible. Adrenaline and its ethers cannot be reversibly oxidized. The probable point of attack of the oxidizing agent in all of these compounds is the two hydroxyl groups on the benzene ring, with the formation of an ortho-quinone. This derivative of adrenalone can be reduced, but, with adrenaline some other part of the molecule absorbs the oxidizing power of the ortho-quinone, resulting in an irreversible reaction. At a pH of 7.4 and in the presence of a third component, adrenaline will act as a cyclic catalyst bringing about the oxidation of adrenaline with molecular oxygen. The essential configuration of the third component has not been determined, but it is prepared by the action of sodium hydroxide on glucose.

Although adrenaline and its derivatives can be oxidized, they do not have a characteristic effect on the platinum electrode and they do not form oxidation and reduction systems in which the oxidized and reduced forms are in equilibrium. It has been shown that the potential of any solution is not determined by the ratio of the oxidized to the reduced form, and that an unstable oxygen addition-product is apparently the most important factor establishing the absolute value of the potential of the solution containing these substances. Oxygen and hydrogen dioxide do not oxidize this series of compounds, they do, however, appear to form some type of addition-product which is stable for many hours, and which has a determining influence upon the equilibrium point of the solutions, which is within limits independent of the ratio of the oxidized to the reduced forms.

This is a preliminary report.

3637

Bacteriologic Observations on Periodic Ophthalmia in Horses.

E. C. ROSENOW.

From the Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minn.

Through the cooperation of Dr. F. Park Lewis of Buffalo, N. Y., I have had opportunity to study the bacteriology of periodic ophthalmia in horses. This disease is said to be the greatest single menace to the horse industry, the world over.

By means of animal inoculation or cultural methods, an organism having specific affinity for the eye has been isolated from the lacrimal sac in 19 of 40 cultures made in 16 affected animals. It has been isolated at least once in 12 of the affected animals, twice in 4 and 3 times in two. The inflammation of the eyes of the 4 animals that yielded negative results had either entirely disappeared or was receding at the time the swabbings were made. It has been isolated from the lacrimal sac in only 2 of 28 normal horses of the herd affected and in no instance of 23 horses cultured remote from the outbreak. It has been isolated from the mucous membrane of the nose in 2 of 6 affected animals, from a sample of water containing yellowish pieces of scum or moss from the shaded side of the tub outdoors, to which the herd had access, and from the water contained in a tub within the stable supplying the drinking water for 10 of the affected animals. Similar cultures from 13 samples of water obtained from brooks, pools, hoof prints, and wagon tracks in the pasture failed to yield the bacillus in a single instance. It was isolated from one specimen of the oats and pea hay which was fed last winter when the epidemic began. It was not obtained in cultures from the feces of 3 nor from the winter coat of hair of one of the affected animals. It has been isolated from the fluid aspirated from the anterior chamber in 4 of 6 acutely affected eyes, and has been isolated in pure culture or in association chiefly with a large diplococcus, from the fluid in the anterior chamber, and emulsions of the exudate covering the iris, the iris, uveal tract, and limbus of an eye of a horse which was removed under chloroform anesthesia for study, early in the third and worse attack.

The suspected organism is a medium-sized Gram negative, motile rod with somewhat rounded ends. It grows rather slowly aerobically on the common culture mediums. On plain or glucose-agar and plain horse blood-agar it produced medium-sized glistening, translucent, non-adherent, yellow to orange colonies with smooth margins, and on potato, a spreading confluent yellow growth. The yellow pigment formation is not as helpful for the isolation of the organism from mixed cultures as it would seem, for in some strains it does not develop until after 48 to 72 hours' incubation and may not appear at all where growth is heavy or where available oxygen is limited. In plain and dextrose broth it produces a moderate, diffuse clouding, most dense in the upper layer and in older cultures, a yellowish pellicle is formed. The pigment remains within the organism; flourescence has not been observed. It produces no change in litmus milk, liquifies gelatin slowly, does not digest Loeffler's blood serum, does not form indol, ferments sugars slightly without gas production, and reduces nitrates to nitrites. Its size and grouping varies considerably, depending upon whether grown in liquid or solid mediums. It usually stains diffusely although polar staining and clear areas sometimes resembling spores occur in a few individual bacilli, especially in old cultures. On agar the organism is quite uniform in size, ranging from 0.5 by 1.2 to 0.7 by 2.5 microns and thread formation is usually absent. In liquid cultures the length of individual rods varies considerably and short threads are sometimes seen. Flagella have not yet been demonstrated. Only a small proportion of the organisms are found actively motile, even in young cultures. It is not acid fast. Young cultures are readily killed by heat and antiseptics, grow rather slowly at 35 to 37° C., and somewhat more quickly at room temperature, the optimal temperature for growth ranges between 25 to 30° C. According to Bergey's classification, the organism belongs to the genus *Flavobacterium,* but it does not conform in all particulars to any of the numerous varieties described as peculiar to this genus.

Great difficulty has been experienced in isolating and maintaining the organism in pure culture. It has happened repeatedly that fishings from well isolated single colonies on platings containing seemingly only characteristic colonies in which smears reveal typical Gram negative bacilli only, and in which young cultures proved to be pure, that Gram positive or negative large or moderate sized cocci, diplococci or diphtheroid bacilli were found in the same culture after prolonged incubation. This has been a puzzling problem and at present it is uncertain whether we are dealing with inadvertent mixtures or mutations. The various forms found in these cultures, especially the large Gram positive or negative diplococcus, resemble very closely the organisms isolated commonly with the bacillus from swabbings of the lacrimal sac and from material obtained from within affected eyes.

When injected in relatively large doses, the organism is virulent for the rabbit, guinea pig, white rat, and the horse. The symptoms and death in each of these species, however, is due apparently chiefly to the presence of toxic material preformed in the broth or to that liberated from the organisms injected. It disappears quickly from the blood after injection in the animal that survives, and is never found in large numbers in blood and other tissues in animals that succumb. It tends to localize in the anterior structures of the eye when injected intravenously.

The reaction in the animals injected in proper dosage is very characteristic, especially in the rabbit. Soon after injection in this animal of 2 to 5 cc. of a broth culture or filtrate per 1000 gm. of body weight, the respirations become increased, weakness becomes manifest, often associated with fine tremors of muscles, especially on exertion, and coincidentally there appears congestion of the vessels of the palpbral and ocular conjunctiva, of the iris, and especially of circumcorneal vessels, and a variable degree of lacrimation. Similar, although milder, symptoms have followed intravenous injections of as little as 0.1 cc. If the animal recovers, the findings in the eye disappear. In one instance turbidity of the fluid in the anterior chamber and other evidence of infection has developed from intravenous injection. Placing the organism in the lacrimal sac with and without injuring the cornea has thus far not resulted in intrinsic infection of the eye. However, a condition strikingly like the specific disease has been produced in several horses and a series of rabbits, by injecting into the anterior chamber, a small amount of pure cultures and filtrates of old cultures. The disease has been induced in both eyes of one horse, by injecting the fluid from the anterior chamber into one, and the emulsion in sodium chloride solution of the membranous exudate covering the anterior surface of the iris into the other. The eye from which this material was obtained was removed on the fourth day of the third typical spontaneous attack. Marked clouding, especially in the lower half of the anterior chamber occurred within 24 hours. The picture in both eyes 10 days since injection is thus far typical of periodic ophthalmia. The bacillus was isolated from the material injected and from the fluid aspirated from the eye receiving the emulsion. Both of these strains have produced the characteristic reaction on intravenous injection in rabbits. Injection of other organisms also isolated have shown little or no affinity for the eye and control injections of sterile broth are without effect. Filtrates of old cultures have been found extremely toxic, especially for the horse. Small amounts introduced into the anterior chamber cause marked clouding of the fluid, circumcorneal congestion, edema of conjunctiva, and lacrimation lasting for a number of days.

It has been found that intravenous or subcutaneous injection of filtrates of exhaust cultures render the eyes of rabbits and horses refractory to the effects of intraocular injections of the organism and filtrates, and in preliminary observations appear to lessen the inflammatory process in the eyes of affected horses. The filtrates are especially toxic for the horse. Two cc. injected intracutaneously and 2 cc. subcutaneously sometimes produces even in adult animals within from 1 to 2 hours, severe chilling, trembling of muscles, increased respiration and general illness in which the animals lie down and not infrequently, manifest unmistakable circumcorneal congestion. The eyes of rabbits that have fully recovered following injection of the living organism or toxic filtrate and the eyes of horses that have fully recovered from the spontaneous disease, appear more refractory to injection of the filtrates than the eyes of normal animals, whereas the eyes in both these species that still show inflammation, are more susceptible than those of normal animals. The skin of the rabbit, guinea pig, goat, monkey, dog and white rat is highly immune to filtrates, even the undiluted filtrate giving little or no reaction, whereas the skin of the horse is extremely sensitive, dilutions as high as 1 to 1000 often giving decided reactions. Presumptive susceptibility tests with filtrates of this organism have been made in a series of normal horses and in affected animals. Great variations in reactions have been observed and a parallelism between the involvement of eye and degree of the skin reactions have been observed in some of the affected herd.

Rabbits and horses are now being immunized in the hope of developing an antitoxic curative serum. The fact that the organism appears to produce a specific toxin measurable by intracutaneous injection, supports the hope that an antitoxin of high titre may be expected and which may perhaps be standardized by measuring its neutralizing power by intradermal injections of appropriate mixtures of toxin and antitoxin.

This is an abstract.

3638

Nitrogenous Constituents of Blood Following Transplantation of Ureters Into Different Levels of Intestine.

JESSE L. BOLLMAN and FRANK C. MANN.

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minn.

In an attempt to study the effect on the kidney of the presence of large amounts of normal urinary constituents in the blood, we transplanted the ureters into different portions of the intestine. A simplified surgical technic was employed and the results were for the most part satisfactory. In most instances the only demonstrable change in the ureter and kidney was slight dilatation of the ureter and pelvis of the kidney, while microscopic examination of the renal tissue revealed but minor changes. In some cases, however, hydroureter and hydronephrosis with subsequent pyonephrosis developed. These cases furnished an interesting comparison to uncomplicated ureteral transplantation.

A single ureter was transplanted into the jejunum, ileum, colon, and rectum in different dogs. When the other kidney and ureter was not disturbed practically no change was observed in the nitrogenous constituents of the blood over a period of several weeks following operation. Bilateral transplantation of the ureters or single transplantation with removal of the kidney on the opposite side produced some very interesting changes in the constituents of the blood. Drainage of the ureters into the rectum produced in most instances a marked rise in the blood urea for from several days to six weeks after operation and a subsequent return of the level of urea in the blood to a figure which is within normal limits. No changes were found in the creatinine, creatine, uric acid, or amino-acid content of the blood. Following ureteral transplantation into the colon there was a similar rise in the blood urea nitrogen without significant changes in the other nitrogenous blood constituents studied. One of these animals maintained a level of urea nitrogen in the blood of slightly more than 400 mg. for each 100 cc. without elevation in the creatinine content of the blood over a period of six days; there was a gradual return to normal about two months after operation.

Ureteral transplantation into the jejunum or ileum elicited a more constant picture. For the first five to seven days there was a rapid rise in the urea nitrogen content of the blood with no appreciable changes in the creatinine, creatine, uric acid, or amino acid content of the blood. In some cases extremely high values for the blood urea nitrogen were obtained. In one animal about two weeks after operation there was 808 mg. urea nitrogen for each 100 cc. of blood. Subsequently the blood urea decreased considerably with the onset of symptoms of uremia, the animals surviving this type of transplantation for from 14 to 20 days. These animals demonstrate the non-toxic nature of urea, since extreme values of blood urea are found without the presence of any symptoms of uremia. The rapid rise of the urea in the blood is in marked contrast with the absence of any appreciable increase in the creatinine content of the blood. This would appear to indicate that creatinine is not readily absorbed from the intestine under these conditions. It was also noted that definite increases in the creatinine content of the blood occurred when the kidney was greatly injured by infection.

This is a preliminary report.

3639

Measurement by Vreeland Oscillator of Minimum Time Required to Stimulate Muscle or Nerve.

J. F. MC CLENDON, WARREN FETTERLY AND PETER J. HINIKER.

From the Laboratory of Physiological Chemistry, University of Minnesota.

Nernst's law of stimulation (stimulus = current$\sqrt{}$time) has been proven for currents from 0.0002 to 0.001 seconds duration, but Lapique showed that a minimum time was necessary and was different for different tissues. A minimal current flowing infinitely which just causes a contraction is called the rheobase, the rheobase is then doubled and the time for stimulation is measured, this time is called the chronaxie. Owing to the uncertainties of the form of many of the currents used in determining chronaxie or to the undesirability of the form of the current (asymptotic) we have adopted a different procedure.

Our studies of the electric stimulation of muscle with alternating current have led us to believe that what is called the "summation of stimulus" is due to the direct current component of the alternating current. The induction coil gives a current having a unidirectional competent, as shown by Washburn, who inserted a silver coulometer in series with the secondary of an induction coil and obtained a deposit of silver.

We have used a Vreeland oscillator, giving a current of pure sine wave form with no direct current component, the frequency of which can be changed by small gradations between 7000 and 300 cycles per second. Since the time of stimulation is one-half of a cycle, the minimum time of stimulation may be determined, our results on the stimulation of gastrocnemius muscle of the frog in Ringer's fluid being 0.000079 seconds. With curarized muscle it was found that the minimum time for stimulation was increased to 0.00009 seconds. The tongue muscle required 0.00013 seconds. The minimal time for stimulation of smooth muscle could not be determined by means of the oscillator because a low enough frequency could not be obtained, but this presents no difficulty since Englemann was able to determine it in 1870.

This is a preliminary report.

3640

An Indirect Calorimeter for the Determination of O_2 and CO_2.

J. F. MC CLENDON AND C. J. VAN SLYKE.

From the Laboratory of Physiological Chemistry, University of Minnesota.

The essential parts of the calorimeter are: (a) a tank containing about 300 litres of water; (b) 2 cylindrical spirometer domes in the tank, the capacity of each being 100 litres and height 1 meter; (c) a tank containing 5 kilos of 4 mesh soda lime connected with each spirometer with a 3-way valve; (d) a glass tube of 2.5 cm. bore shaped like the Greek letter π, one end of the horizontal portion being the mouth piece, the other closed by a rubber stopper at the moment the experiment is to begin, and the 2 vertical portions fitted with 4 cm. lengths of Visking sausage casings (to act as valves) and connected by rubber tubing to the 3-way valves. Each spirometer dome is counterbalanced by a weight fastened to a bicycle chain passing over a large bicycle sprocket wheel. The thickness of the wall of the dome is such that the weight of water displaced equals the weight of the chain. A meter stick is used as a scale and each mm. corresponds to 100 cc. A thermometer is inserted in each spirometer dome. The tube leading from the 3-way valve up through the water into the spirometer has a large enough surface to bring the air passing through it to the temperature of the water and this tube in spirometer No. 1 contains a wick to saturate the air with moisture.

In operation, spirometer No. 1 is filled with outdoor air (the CO_2 of which is considered negligible), the temperature recorded and spirometer No. 2 left empty, the mouthpiece adjusted to the patient and the breathing watched. At the end of an expiration the rubber stopper is inserted and stop watch started. Air now passes from spirometer No. 1 into the lungs and out into spirometer No. 2. At the end of about 10 minutes or until spirometer No. 1 is nearly empty, but at the end of an expiration, the rubber stopper is removed and stop watch stopped. The reduction in spirometer No. 1 and volume and temperature in spirometer No. 2 are recorded, spirometer No. 1 is emptied to the outside and the 3-way valves turned to connect 1 and 2. By placing a 300 gm. weight on 2, the air slowly passes through soda lime into 1, where its volume and temperature are recorded.

The CO_2 is the difference in volume of the air after and before it passed through the soda lime. The O_2 absorbed by the patient is the difference between the volume of the air after passing through the soda lime and that passing from spirometer No. 1 into the lungs of the patient. In order to verify the complete absorption of CO_2 it is merely necessary to pass the air through the soda lime into 1, then back into 2 and see that the volume is constant.

In the practical working of the apparatus it is not only necessary to read the temperature in each spirometer but also to see that the temperature of the air in one spirometer is the same as that in the other and does not change appreciably in 10 minutes. The water is of sufficient volume to absorb the heat from the lungs and soda lime without appreciable rise in temperature, but when the dome of a spirometer is filled with air, heat exchange through the thin wall of the spirometer must be minimized. The wall must be thin as its displacement equals the weight of the chain, but is better to be of heat insulating material. Oil on the water outside the dome is desirable. Having the dome in a space at the same temperature as the water and saturated with moisture would be desirable.

This is a preliminary paper.

3641

Hydrochloric Acid and Total Chlorine Content of Pure Gastric Juice Produced After Histamine Injection.

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From the Medical Service, University Hospital, Minneapolis.

The acid curve obtained from a fractional analysis of an ordinary test meal is at present in a stage of reinterpretation. This new interpretation is based on Boldyreff's theory about the automatic regulation of the acidity in the stomach.[1] Clinically this theory is being supported chiefly by work from Guy's Hospital (Bolton and Goodhart;[2] Baird, Campbell and Hern;[3] Ryle[4]). The chief point in the interpretation is that the total chlorides of the gastric content represent a true expression of the acid production in the stomach. The difference between the curve expressing the free hydrochloric acid and the total chloride curve, therefore, according to this theory represents the extent of neutralization of acid either by combining with protein or by neutralization with regurgitated alkaline duodenal content, particularly pancreatic juice. The value of this theory evidently depends upon several factors. One important point to be ascertained is whether in the pure gastric juice chlorides are present in other forms than HCl. Other analyses indicating a fair agreement between hydrochloric acid and total chlorides are not entirely satisfactory in this connection. Schoumow-Simanowsky,[6] for instance, used litmus as indicator in her titration for free acid, Rosemann[7] used phenolphthalein.

We have determined the total chlorides and the free hydrochloric acid of the gastric juice produced after subcutaneous histamine injection (Lim, Matheson and Schlapp[5]). We have used normal individuals as well as hospital patients. The standard dose of histamine has been three milligrams ergamine (Burroughs Wellcome). The stomach was first carefully washed out and emptied. Samples were withdrawn every fifteen minutes following the injection of the histamine. Dimethyl-amino-azobenzene was used as indicator in determining the free acid. Van Slyke's method was used for the chlorine determinations. On pure hydrochloric acid solutions identical figures were obtained by the two methods. Chart 1 is presented to indicate the close correspondence between the free acid and the total chlorides. Shaded portion represents limits for free HCl in 80 per cent of normal people. (Bennett and Ryle.[11]) Chart 2 shows all way through a greater difference between the free acid and the total chlorides, the curves, however, being regular in shape. We believe this difference is due to combining of acid with mucus. Chart 3 shows marked and irregular neutralization by regurgitation. The regurgitation was here evidenced by the staining of the stomach content with bile.

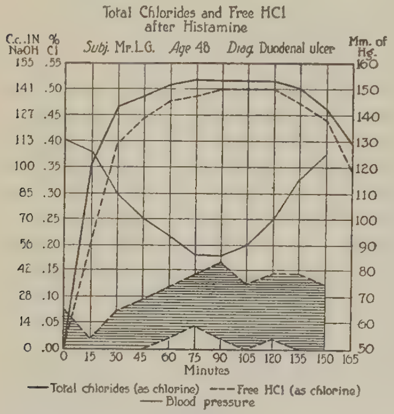

CHART 1.

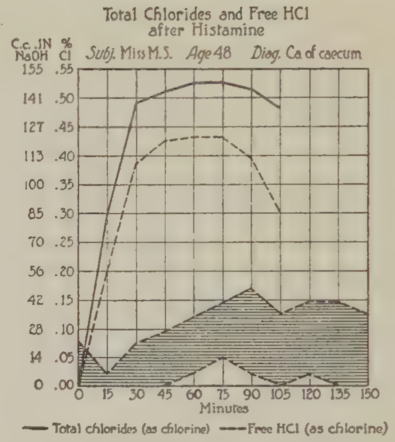

CHART 2.

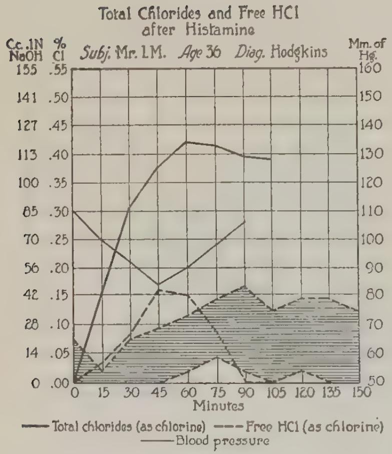

CHART 3.

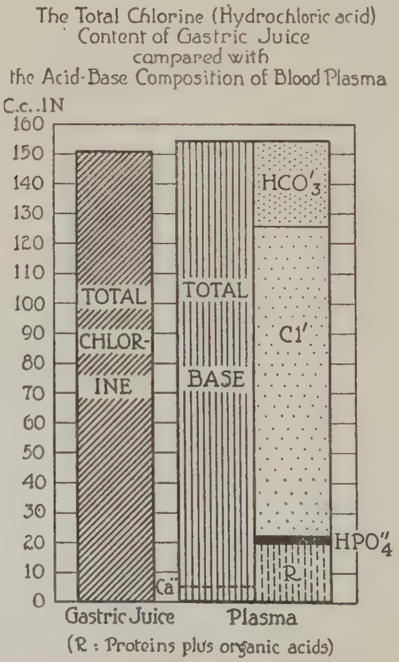

CHART 4.

From the close correspondence between the two curves in the first chart as well as from the same correspondence in other curves not reproduced in this preliminary report, we conclude that the normal stomach juice contains only insignificant amounts of chlorine, if any, in other forms than hydrochloric acid.

Attempts to correlate the total chlorides of the gastric juice with the chlorine content of the blood plasma failed to reveal any correlation, while there is an almost complete correlation between the highest chlorine figure obtained for the gastric juice and the total base of the blood plasma. (Chart 4, the plasma diagram from Gamble, Blackfan and Hamilton.[8]) This correlation is believed to be of definite significance. The gastric juice is therefore believed to fall in the same group of body secretions as milk and bile, this group having a depression of the freezing point not greater than that of the plasma, in this respect differing from the urine, where a concentration beyond the Δ of the plasma takes place. It is well known (Carlson[9]) that the fish shows a hydrochloric acid concentration in its gastric juice far above the concentration in mammals. This, however, does not break the rule since the freezing point depression of the plasma, and consequently the fixed base in the different species of fish, are correspondingly increased (for reference to the osmotic pressure in fish see Schmidt-Nielsen[10]).

This is a preliminary report.

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3642

Effect of Pregnancy Upon the Emptying of the Gall Bladder.

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It has been definitely established that the feeding of certain foods, particularly egg yolk and cream will cause the gall bladder to empty; the emptying is due to a contraction of its intrinsic musculature. The mechanism involved in the contraction of the gall bladder has not been determined. It was felt, however, that should a chemical or a nervous mechanism be responsible, it could be determined by feeding pregnant animals egg yolk and cream, and observing the condition of the fetal gall bladder at a time when the maternal gall bladder was empty.

Accordingly, a group of pregnant dogs and guinea pigs were fed egg yolk and cream, the usual test-meal. After a period equivalent to that in which the gall bladder empties completely in the nonpregnant guinea pig and partially in the nonpregnant dog, the animals were etherized, the gall bladder explored under sterile technic and one or more of the fetuses removed. However, the gall bladder of the mother, explored at a time after feeding equivalent to that in which the nonpregnant animal's gall bladder is partially or completely emptied, showed little or no change in size. Observations were made on a large series of pregnant dogs and guinea pigs, but the gall bladder was not observed to empty following the ingestion of egg yolk and cream except in two dogs in which the gall bladders were examined 8 and 10 hours respectively after the taking of the fat meal, when slight but definite emptying was noted.

After we discovered that the gall bladder of the pregnant dog and the guinea pig did not empty as in the nonpregnant animal, we obtained a large number of spermophiles (common striped gopher) including males, nonpregnant females and females during various periods of pregnancy. It was found that the gall bladder of the male emptied approximately 4 hours following the ingestion of egg yolk and cream. The gall bladder of the nonpregnant female emptied similarly. The gall bladder of the pregnant female, on the other hand, was not seen to empty completely and partial emptying occurred in only a few instances; this occurred in early pregnancy only. The degree of emptying seemed to bear a direct relationship to the time of pregnancy.

The reason why the gall bladder of the pregnant animal does not empty following the ingestion of a meal of fat has not been definitely determined. However, it has been observed that the fat leaves the stomach much more slowly in the pregnant than in the nonpregnant animal, and that while the lacteals and the lymph nodes of the nonpregnant animal are usually perfectly white at the time the gall bladder is empty, the lacteals of the pregnant animal never appear to become well delineated or markedly distended. It would appear that the control mechanism of the gastro-intestinal tract is retarded in pregnancy, and thus the gall bladder either does not empty or is greatly delayed in emptying. Increase of the intra-abdominal pressure produced by the growing fetuses should be considered as a possible cause for the delay or the prevention of emptying the gall bladder following the ingestion of fat, yet this is not of great importance, as is shown by the fact that the gall bladder of a spermophile may not empty, or may empty only slightly, when the fetuses are no larger than the fecal material in the large intestine. In this connection it should be noted that in the two instances in which partial emptying of the gall bladder was noted in the dog, the gall bladders of the fetuses were also partially empty and dark-colored bile was found in the duodenum, as contrasted with the distended gall bladder and the duodenum free from bile, observed in the large number of fetuses removed from dogs whose gall bladders did not empty in the shorter period of time following the ingestion of egg yolk and cream. It is suggested that the failure of the gall bladder of the pregnant animal to empty following the ingestion of food may bear some relation to the high incidence of gall stones following pregnancy in the human being.

This is a preliminary report.

3643

Emptying of Gall Bladder and Mechanism of Common Bile Duct of Guinea Pig.

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The observations of Boyden[1] that a meal of fat is followed by partial or complete emptying of the gall bladder, proved definitely that the viscus emptied. The experiments of Boyden,[1] Whitaker,[2] Higgins and Mann,[3] and Hamrick[4] demonstrated that this emptying of the gall bladder was due to the contraction of its own intrinsic musculature. Other observers, Kodama,[5] Graham,[6] and Burget[7] have failed to empty the viscus by contracting its own intrinsic musculature. It should be noted, especially in relation to the experiments of Burget,[7] that whenever contraction of the gall bladder and expulsion of bile has been demonstrated it does not occur under an anesthetic.

We have found that under certain definite conditions the gall bladder of the guinea pig will contract and completely empty its contents within a brief period. A description of this simple experiment is given, not because it may bear any direct relationship to the normal physiologic mechanism of the gall bladder, since we have previously demonstrated that the viscus of this animal will empty following a meal of egg yolk and cream, but because it is possible to use this experiment as a classroom demonstration. Even an amateur in laboratory technic can demonstrate the emptying of the gall bladder by the contraction of its own musculature. The routine of the experiment is as follows:

A guinea pig which has been fasted only a few hours, is killed quickly with chloroform. The abdomen is opened immediately and the duodenum exposed. Gentle traction is made on the duodenum, and the entire biliary tract exposed. The gall bladder will be found partially or completely filled with bile. Gentle stroking of the gall bladder with thumb forceps or the edge of a knife will cause it to begin to contract immediately and in from three to seven minutes its entire contents will be forced into the duodenum. As the traction on the duodenum has raised the point of discharge of the bile above the level of the gall bladder, the bile is discharged against gravity. While contracting, the surface of the gall bladder becomes covered with bleb-like projections which appear to be small herniations of mucosa forced out between breaks in the musculature of the wall of the gall bladder. It is rare that the gall bladder of the guinea pig will not empty completely under the conditions described. It can also be made to empty, with the animal under light anesthesia, by stimulation with electricity. It usually does not empty as quickly with the anesthetic as immediately after the animal is killed. We have occasionally seen the contraction and partial emptying of the gall bladder of other species, as the cat and dog, following stimulation of the wall of the viscus when the animal had been killed quickly.

One of the most interesting points in connection with the biliary apparatus of the guinea pig is a peculiar muscular organ situated at the duodenal end of the common bile duct. This was shown by us several years ago in a drawing of the biliary apparatus of the guinea pig. The structure is formed by a definite dilatation and partial sacculation of the common bile duct and contains a thick layer of smooth muscle almost as great as the muscularis of the duodenum. This organ pulsates during the emptying of the gall bladder. However, the emptying of the gall bladder is not necessarily dependent on this peculiar structure because, when a cannula is inserted in the common bile duct on the hepatic side of this structure, and the gall bladder stimulated, it will still contract and produce considerable pressure (80 mm. water) in a straight glass manometer. No similar structure has been found in any of the many other species of animals examined. A complete report on the mechanism of the biliary tract of the guinea pig will be made later.

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3644

Blood Sugar Level and Activity in Rats.

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From the Departments of Physiology and Psychology, University of Minnesota.

The primary problem in the study of activity is to discover those internal factors and conditions which stimulate an animal to become active. Since the energy for muscular contraction comes from the oxidation of glucose it is conceivable that a relation exists between blood sugar and activity.

In studying this relationship 45 male albino rats, 5 months old, were used; these were divided into 2 groups of 19 and 26 animals respectively. Their activity was recorded in revolving drums of the type used by Slonaker, Hoskins, and others who have worked on activity. The animals lived in these cages for six weeks; they were fed 30 cc. of bread and milk at a regular hour each day, and daily turns of each cage recorded. Temperature and light conditions were constant. The animals received no food during the 24 hours just preceding blood sugar but they remained in the activity cages until about 2 hours before their death. The activity measure which was chosen as best representing the level of an animal's activity at the time blood sugars were done, was the median of his last five days runs.

The animal was sacrificed for making the blood sugar analysis. The sugar determinations were made according to Folin's copper reduction method as revised in his 1926 articles. Sugars varied within a range 66 to 125 mg. per 100 cc. The average for all animals was 95 mg.

Correlations between blood sugar and activity ran thus:

Group I	19 cases	Blood sugar and activity	—.21 ± .155
Group II	26 cases	Blood sugar and activity	—.05 ± .139
All animals	45 cases	Blood sugar and activity	—.21 ± .10

These correlations, although so near zero as to indicate little more than a chance relationship, still are consistent as to sign and two are of the same magnitude. They seem to indicate that high activity is accompanied by low blood sugar, and low activity by high blood sugar.

To test this point further the average activity for animals having blood sugars of 100 mg. and above was compared with that for animals having sugars below 90 mg. The results are:

	No. of cases	Av. sugar	Av. activity in rev. per day	P. E. Av.
Blood sugar 100 and above	19	112	3468	±263
Blood sugar below 90	16	82	5749	±773

On the average those having low sugars are more active than those having high sugars. The two most active rats had sugars of .92 and .91. The difference in activity between the low and high sugar groups is such that one can predict, by the ordinary statistical methods, that the difference in activity will lie in favor of those animals having lower sugars in 97 per cent of the future repetitions of this experiment.

The evidence, therefore, seems to point toward an inverse relationship between activity and blood sugar. Physiologically this probably means that the more active rat uses up his available sugar at a more rapid rate than his less active brother. Whether or not by inducing a high level of sugar in all animals, one would reduce activity is a problem to be attacked soon.

This is a preliminary report.

New York

3645

On Inhibiting Effect of Acetates and Acetic Acid on Living Cells of Nitella.

MARIAN IRWIN.

From the Laboratories of the Rockefeller Institute.

When living cells of *Nitella* were exposed for 10 minutes (1) to M/150 acetate buffer mixture at pH 5.5 or at pH 4.8 (consisting of a mixture of acetic acid and sodium acetate), and (2) to acetic acid at pH 4.8 or at pH 4, the pH value of the sap in the vacuole was found to decrease (depending on the supply of acetic acid in the external solution). If such cells were now placed for ½ minute in the solution of brilliant cresyl blue, made up with M/150 borate buffer mixture at pH 7.85, the rate of penetration of dye into the vacuole was found to decrease, as compared to that of control cells which were placed directly from the tap water into the same dye solution. At pH 4.8, the acetate buffer mixture brought about much greater decrease in the rate of penetration of dye into the vacuole than acetic acid. This may be due to the presence of sodium and a greater supply of acetic acid in the case of the former.

When cells were placed in M/150 sodium acetate solution for 10 minutes the pH value of the sap was found to remain unchanged, but when such cells were placed in the dye solution, as before, the rate of penetration of dye into the vacuole was found to decrease considerably.

From these results we may assume that the inhibiting effect of acetate buffer mixture may be due partly (1) to the effect of acetic acid on the protoplasm (either due to a specific effect of the acid or to the entrance of acetic acid as undissociated molecules and its subsequent dissociation whereby lowering the pH value of the protoplasm corresponding to the lowering of the pH value of the sap in the vacuole); and (2) partly to the effect of sodium acetate on the protoplasm.

When living cells were exposed to these solutions as before and were placed in the dye solution made up with M/150 phosphate buffer mixture at pH 7.85, the rate of penetration of dye was found to decrease in the case of the acetate buffer mixture and acetic acid, but it did not change from that of the control in the case of the sodium acetate. The inhibiting effect of acetic acid on the protoplasm might have persisted in this case because the acetic acid in the vacuole served as a reserve supply by diffusing into the protoplasm after the cells had been transferred from these solutions into the dye solution, while that of the sodium acetate did not persist because there was no storage of sodium acetate or acetic acid in the vacuole.

Some of these experiments seem to indicate that though a decrease in the rate of penetration of a basic dye into the vacuole may take place at the same time the pH value of the sap is decreased, such results do not necessarily discredit the theory that a basic dye enters the vacuole much more readily in form of free base than in form of salt. If we assume that the inhibiting effect of acetic acid on the protoplasm exceeds the accelerating effect on the vacuolar sap, and that the rate of penetration of dye into the vacuole is controlled in this case by the diffusion of dye in form of free base from protoplasm into the vacuole, then a decrease in the pH value or some other alteration in the protoplasm corresponding to a decrease in the pH value of the sap might very well bring about a decrease in the rate of penetration of the dye into the vacuole.

3646

A Method of Proteolytic Enzyme Titration.

FREDERICK L. GATES.

From the Rockefeller Institute for Medical Research.

In 1906 Müller and Jochmann[1] proposed a simple method of determining the presence of proteolytic enzymes in biological fluids by erosion of the surface of a Loeffler serum plate under a drop of the fluid. Later this technique was modified by the use of a gelatin plate dyed with carmine, but at best it was a + or — method, probably because the substrate was not sufficiently uniform to permit quantitative estimates of enzyme action.

If a photographic plate, or film, is fogged on both sides by equal exposures, is developed, fixed, washed, and dried, it presents a gelatin surface and texture of sufficient uniformity for quantitative tests. Proteolytic enzymes erode the surface and free the included silver, so that the light transmission of the exposed area increases as digestion proceeds, and can be estimated relatively by colorimetry, or directly by a calibrated thermopile and galvanometer. The method is as follows:

Small paraffined chambers are filled with trypsin or pepsin solution so that the meniscus projects above the rim. Face down over each chamber is placed a small square of the photographic plate, which has been previously brought to the proper pH concentration in phosphate buffer solution and allowed to dry without rinsing. Due to the inverted position of the gelatin layer the products of proteolysis and the released silver fall away from the surface, and interfere less with the progress of the reaction than as if they accumulated there. Temperature is carefully controlled, and plates are removed at timed intervals, rinsed quickly in cold water, immersed in a bath of the opposite reaction to arrest enzyme action, and dried rapidly before a fan.

The successive plates will be found to present areas of decreasing density. A quantitative estimate of the progress of the reaction is made with a Klett or other Duboscqre type colorimeter. On one side, under the cup stand, is placed a square of the photographic plate from which the silver emulsion has been removed. The gelatin-silver suspension from this piece of plate, dissolved off in hot 25 per cent glycerine solution (to maintain the silver longer in even suspension) is poured into this cup, as a variable comparison standard against which the eroded areas of the experimental plates, under the other cup (filled with 25 per cent glycerine solution) are read. A piece of the same plate which has been soaked in the enzyme solvent and dried is used for the control, or initial density reading.

The match is exact, since specimens of the same materials intercept the light on both sides of the colorimeter, and readings may be made repeatedly within 1 or 2 per cent. Preliminary titrations of trypsin solutions have given exponential curves, and seem to indicate a mononuclear reaction. The method is perhaps not as sensitive as the viscosity method of Northrop and Hussey,[2] but with more concentrated enzyme solutions it gives rapid determinations with an error of less than 5 per cent.

This is a preliminary report.

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[1] Müller, Edw., and Jochmann, G., *Munch. Med. Woch.*, 1906, xxix, 1393.

[2] Northrop, J. H., and Hussey, R. G., *J. Gen. Physiol.*, 1923, v, 353.

3647

Action of Narcotics on the Ameba by Means of Microinjection and Immersion.

STANISLAW HILLER.* (Introduced by Robert Chambers.)

From Cornell University Medical College, New York City.

Comparative studies were made on the influence of the narcotics ethyl alcohol, chloretone, ether, and chloroform on the protoplasm of *Amoeba dubia* with special reference to their action on the plasmalemma.

Immersion Experiments: Very weak concentrations (subnarcotic) cause the amebae to spread out and continue their movements in an expanded condition. This may be an effect of the lowered surface tension. Ether in its narcotic concentration (approximately 2.0 per cent) produces a reversible gelation accompanied by the cessation of all movements. Lethal concentrations of all the narcotics cause the ameba to round up, followed by a sinking of its granules and a disintegration of the plasmalemma.

Injection Experiments: No narcotic effect is produced by injection into the interior of the ameba. Chloretone in all concentrations increases the fluidity and streaming movements of the interior. Eighty per cent alcohol produces a coagulation which is localized and reversible when small amounts are injected and irreversible with large amounts. Chloroform and ether when injected into the cytoplasm form spherical drops which go into solution more rapidly than when injected into water. The protoplasm is irreversibly coagulated in the environment of the dissolving droplet. Chloroform is more potent than ether in causing coagulation. The irreversible coagula produced by the alcohol, chloroform and ether are pinched off by the living portion of the ameba.

Local Application of the Narcotic on the Exterior of the Ameba: With saturated chloretone solution and 95 per cent alcohol no local effects were obtained. All the pseudopodia are withdrawn and the ameba was converted into an actively moving limax form.

Concentrated, aqueous solution of ether produces a local blister on the plasmalemma with a coagulation of the cytoplasm at the base of the blister. A drop of pure ether dissolves the plasmalemma on remaining in contact with it for five seconds or more. This occurs most rapidly at the tip of a newly forming pseudopodium. As the ether droplet goes into solution there appears in its place a wrinkled pellicle enclosing several highly refractive globules. Both the pellicle and the globules are insoluble in water and in cold 95 per cent alcohol.

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*Fellow International Education Board, Rockefeller Foundation.

Chloroform similarly but more rapidly dissolves the plasmalemma. A small drop on touching the plasmalemma quickly passes through it and the endoplasm in its vicinity becomes coagulated.

An interesting phenomenon which possibly is related to the problem of pseudopodial formation occurs when chloroform is allowed to diffuse against the ameba from the tip of the micropipette. A local elevation of the pellicle takes place, into which the granular endoplasm flows. As the pipette is gradually withdrawn the incipient pseudopodium enlarges and extends in the direction of the moving pipette. In this way the entire ameba can be made to move to all appearances in a normal fashion along any path taken by the tip of the pipette.

I wish to thank Dr. Robert Chambers most sincerely for his kind help in this work.

3648

Influence of Vegetative Nerves Upon Distribution of Arsenic After Salvarsan Injections. II. Pilocarpinized Animals.

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A previous publication[1] gave a comparison between the distribution of arsenic after intravenous injection of arsenicals in normal animals[2] and in animals under the influence of epinephrin. When the involuntary nervous system is under the influence of epinephrin, almost 5 times as much arsenic is retained in the blood stream and only a small part of the arsenic normally distributed to the brain and liver reaches those organs. The general aspect of these tests led to the assumption that epinephrin inhibits the normal distribution of arsenic into the body organs.

In a second series of tests, 2 groups of rats were given subcutaneous injections of pilocarpin previous to the intravenous injections of salvarsan, neosalvarsan and silversalvarsan. 160 mg. of salvarsan per kg. body weight was the dosage used for the normal rats and the pilocarpinized rats were given 80 mg. per kg. body weight. The figures for neosalvarsan and silversalvarsan are similar to those reported for salvarsan and will be given in detail in a later paper. The rats receiving pilocarpin were divided into two groups. One group received one injection of 5 mg. of pilocarpin 1 hour before the injection of the arsenical while, in the other group, four doses of 5 mg. each of pilocarpin were given 72, 48, 24 and 1 hour before the intravenous injections of the same arsenical preparations. The results for the two groups, as shown in the chart below do not differ greatly. Minor differences will be discussed in a later publication.

TABLE I.

*Average arsenic content of organs after intravenous injection of arsenicals preceded by subcutaneous injection of pilocarpin.**

Interval	Salvarsan alone	Salvarsan preceded by 1 previous pilocarpin	Salvarsan preceded by 4 previous pilocarpins
LIVER.			
Immediate	9.33	70.87	77.82
5 minutes	33.84	87.27	76.89
20 minutes	96.02	86.79	68.11
40 minutes	39.52	83.26	102.73
BLOOD.			
Immediate	14.75	204.19	147.25
5 minutes	42.21	157.72	119.29
20 minutes	13.72	80.18	80.27
40 minutes	13.69	106.93	47.17
BRAIN.			
Immediate	39.12	1.126	0.878
5 minutes	5.51	1.236	1.129
20 minutes	1.90	0.298	0.593
40 minutes	2.66	2.148	1.439

*Arsenic figures are given in mg. per 100 gm. dry specimen. Each figure represents an average of five determinations.

Since the pharmaco-dynamic action of pilocarpin is the reverse of that of epinephrin, one would expect to have found an even greater distribution of arsenic into the organs of pilocarpinized animals than in normal rats. Actually there is a greater retention of arsenic in the blood and the character of the curve for the arsenic content of the blood differs considerably from the normal. Usually, the arsenic content of the blood first decreases due to the rapid distribution and deposition of arsenic into the body organs, and then increases between the fifth and tenth minutes after the injection of salvarsan as the metal is released from the organs where it was first deposited. The arsenic then gradually passes from the blood into the liver where it is accumulated. These findings indicate a distinct participation of the liver in the process of arsenic distribution.

This mechanism is entirely changed in the pilocarpin tests. The arsenic figures for the liver are higher than normal, but the concentration in the liver is much less than that in the blood. The arsenic content of the liver remains practically constant throughout the experimental period. The tests show that much smaller quantities of arsenic are deposited in other organs such as brain and spleen. Therefore, starting immediately after the injection, comparatively large quantities of arsenic reach the liver and remain deposited in that organ. No increase in the concentration takes place later because no arsenic is released from other organs.

Summary. Studies on arsenic localization and distribution in rats under the influence of pilocarpin show, as do previous tests with epinephrin, how essential a normal state and a normal, undisturbed activity of the vegetative nerves are for the distribution of arsenic therapeutically administered in a mammalian body. They demonstrate that one has to deal, not with a particular state of vascular or capillary permeability, that regulates and controls arsenic distribution, but with a rather complicated mechanism. Every disturbance of the vegetative nerves alters the character of arsenic distribution, regardless of the type of change actually made in the vascular or capillary permeability. This gives evidence of the important part which has to be attributed to the vegetative nervous system for the cooperative synergistic action of the body organs participating in, and responsible for, the distribution of arsenic after the injections of salvarsan.

This is a preliminary report.

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[1] Müller, E. F., Myers, C. N., and Marples, E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 689.

[2] Fordyce, J. A., Rosen, I., and Myers, C. N., *Am. J. Syph.*, 1924, viii, 377.

3649

Further Observations on Individual Differences of Human Blood.

K. LANDSTEINER AND PHILIP LEVINE.

From the Laboratories of the Rockefeller Institute for Medical Research.

In a previous communication[1] we described an agglutinable factor (M), independent of the blood groups and present in many but not in all human bloods. A somewhat higher incidence of M among colored than white individuals, as indicated by our first results, was confirmed by examination on a larger scale; among 902 white individuals 165 (18.3 per cent), and among 338 colored 95 (28.1 per cent) whose blood reacted negatively. A differentiation of bloods (in the same group) lacking or possessing the factor M, was possible also with dried specimens.

The heredity of the property M was studied in more than 100 families. The results are in keeping with the assumption that M is inherited as a mendelian dominant. In the following table the families are arranged in 3 classes according to the presence or absence of the factor M in the parents, and its incidence among the offspring is given.

TABLE I.

Number of families	65		39		3	
Parents	+	+	+	—	—	—
Number of children	252+	25—	114+	52—	0+	11—

It may be mentioned incidentally that the character[2] A^1 present in most bloods of group A, seems to be an inheritable quality. For, in our tests, if the property was not or slightly developed in the blood of both parents, this, as a rule, was also the case for the children.

With a method similar to that employed for the detection of M, namely, suitable absorption of certain rabbit immune sera for human blood, two other agglutinable qualities were found which may be denoted as N and P. These reactions, further distinguishing individual human bloods, vary in intensity from very strong to weak or negative.

The reactions for N seem to be strong in most cases where M is absent. The property M could be demonstrated also in the blood of eight chimpanzees examined, but was not found in the blood of five gibbons.

Strong reactions for P were considerably more frequent with the blood of colored than white individuals; similar results were also obtained with suitably absorbed normal rabbit and horse sera.

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[1] Landsteiner, K., and Levine, P., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 600.

[2] For the terminology see Landsteiner, K., and Witt, D. H., *J. of Immunol.*, 1926, xi, 221.

3650

Reversion of Avirulent "Rough" Forms of Pneumococcus to Virulent "Smooth" Types.

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From the Hospital of the Rockefeller Institute for Medical Research.

The fact that virulent, type-specific, capsulated, "S" forms of pneumococcus can be degraded to avirulent, non-type-specific, unencapsulated, "R" forms is well established. (Friel,[1] Stryker,[2] Griffith,[3] Amoss,[4] and Reimann.[5]) The reverse process, however, the conversion of avirulent, "R" forms into virulent, "S" forms, has been a matter of some controversy.

Felton and Dougherty,[6] by means of an automatic transferring device in a milk-containing medium, were able to restore virulence to a strain of pneumococcus which had become completely avirulent. They employed pure line strains derived from single cells, and report that some cultures showed a maximal increase in virulence while others remained avirulent. Their investigation preceded the recognition of "R" and "S" forms of pneumococcus. Griffith[3] states that "R" strains may revert in all respects to the "S" type or remain unchanged after many generations in subculture or plain blood broth. Single cell cultures, however, were not employed. Reimann[5] reports that he was unable to enhance the virulence of an "R" strain derived from Type I even after 105 mouse passages. Levinthal[7] was able to effect the transformation by growing "R" forms in serum broth at 25° C. and subsequently passing the cultures through mice.

For bacteria other than pneumococcus the same differences of opinion appear to exist. (De Kruif,[8] Schütze,[9] Savage and White.[10]) Recently, however, Jordan[11] has demonstrated the interconvertibility of "R" and "S" forms of *B. paratyphosus B,* and Soule[12] has shown the reversibility of the dissociated form of *B. subtilis.*

In the present investigation pure line strains derived from single cell cultures of pneumococcus were employed. The single cells were isolated by the method of Avery and Leland.[13]

I. Reversion by Animal Passage. With eight pure line strains of "R" pneumococci, derived from Types I, II, and III "S", it has been found possible in all cases, with one exception, to effect reversion to the "S" form by means of mouse passage. The single exception was that of an "R" form derived from Type I, which, under prolonged cultivation, appears to have lost permanently its ability to revert. Upon reversion the organisms acquire all the properties of the "S" form, including virulence, so that 10^{-6} cc. of the reverted culture regularly kills white mice, while 0.5 cc. of the original "R" culture fails to do so.

II. Reversion by Growth in Anti "R" Sera. Soule,[12] investigating the microbic dissociation of *B. subtilis,* states that, "by the incorporation of "S" or "R" immune sera in fluid media "R" forms can be obtained from "S" forms and "S" forms from "R" respectively.

Since it is known that "S" types of pneumococcus when grown in homologous immune (anti "S") sera change to the degraded "R" forms, it was thought of interest to determine whether the reverse process would occur if "R" organisms were grown in media containing anti "R" sera.

Accordingly, a series of pure line "R" cultures were grown in broth to which the serum of rabbits immunized to "R" forms was added. By this method six "R" strains derived from Types II and III have been caused to revert to type-specific "S" forms. So far, however, it has not been found possible to effect this change with those Type I R strains which were selected. As in the method of animal passage, reversion by cultural methods is accompanied by the acquisition of maximal virulence, the ability to form capsules, and to elaborate the specific soluble substance upon type specificity depends.[14]

In all cases in which the transformation has been effected, reversion has invariably been toward the specific type from which the "R" form was originally derived. Thus far the reversion from "R" to "S" pneumococcus by *in vitro* methods has only succeeded when the serum employed contained anti "R" antibodies. The nature of the stimulus to reversion is at present being further investigated.

This method, then, in certain instances at least, represents a simple *in vitro* means of enhancing virulence and restoring type-specificity to avirulent "R" forms of pneumococci.

This is a preliminary report.

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[1] Friel, A. R., *Pub. S. A. Inst. Med. Res.*, 1915, No. 5.

[2] Stryker, L. M., *J. Exp. Med.*, 1916, xxiv, 49.

[3] Griffith, F., *Rep. Pub. Health, Ministry of Health*, 1923, No. 18, (1).

[4] Amoss, H. L., *J. Exp. Med.*, 1925, xli, 649.

[5] Reimann, H. A., *J. Exp. Med.*, 1925, xli, 587.

[6] Felton, L. S., and Dougherty, K. M., *J. Exp. Med.*, 1924, xxxix, 137.

[7] Levinthal, W., *Klin Wochschr.*, 1926, ii, 2020.

[8] De Kruif, P. H., *J. Exp. Med.*, 1922, xxv, 561.

[9] Schütze, H., *J. Hyg.*, 1921, xx, 330.

[10] Savage, W. G., and White, P. B., *Spec. Rep. 91, Med. Res. Council*, 1925.

[11] Jordan, E. O., *J. Am. Med. Assn.*, 1926, lxxxvi, 177.

[12] Soule, M. H., *J. Bact.*, 1927, xiii, 41.

[13] Avery, Roy C., and Leland, Stanley J., *J. Exp. Med.*, 1927, xlv, 1003.

[14] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, xxvi, 477. Heidelberger, M., and Avery, O. T., *ibid.*, 1923, xxxviii, 73; 1924, xl, 301.

3651

Further Experiments on the Transplantation of Neural Crest (Mesectoderm) in Amphibians.

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In previous experiments[1] the author has shown that when mesectoderm was excised in the cranial regions of early stages of embryos of *Amblystoma punctatum* deficiencies were produced in the branchial skeleton, Meckel's cartilage, palatoquadrate bar, anterior trabeculae and loose mesenchyme in the head. It was further found[2] that when groups of mesectoderm cells were transplanted to new positions on the side of the body they differentiated into cartilages in the presence of the strange mesentoderm (mesoderm) of the somites and in the absence of mesentoderm of the visceral arches.

A similar study has been extended to the Anurans. In *Rana palustris* the author reported[3] a migration of bands of mesectoderm over the mesentoderm of the visceral arches from the dorsal part of the cranial portion of the neural folds similar to the condition found in Amblystoma. The migration begins before the closure of the neural folds—an earlier stage than in Amblystoma. It was found in those studies[3] in *Rana* that when the mesectoderm was removed deficiencies were produced in the visceral skeleton on the operated side.

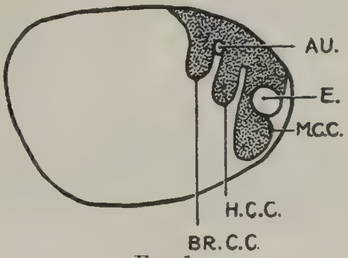

FIG. 1.

Camera-lucida drawing of *Rana palustris* embryo just before closure of neural folds showing extent of migration of mandibular (M. C. C.), hyoid (H. C. C.), and branchial (BR. C. C.) mesectoderm (stippled) at time of operation. AU., auditory placode. E., optic vesicle. X 15.

Further tests of the ability of the mesectoderm to form cartilages in the anurans have been made by a series of transplantation experiments upon early stages of *Rana palustris* in which one or more bands of these cells have been placed in new positions on the side of the body. The relative growth of the bands of mesectoderm at the time of operation is shown in Fig. 1. This is just before the closure of the neural folds and seems to be the most feasible time for removing the mesectoderm for transplantation. Very soon after this stage the mesectoderm over the branchial region splits into 4 bands, 1 for each branchial arch. Then the mesectoderm rapidly migrates further ventrally upon the surface of the visceral arches and soon takes a position upon their medial surfaces where it forms cartilage.

A large area of ectoderm over the branchial and mandibular regions was carefully excised so as to expose intact the bands of mesectoderm (neural crest) as they lie upon the side of the brain and upon the mesentoderm of the visceral arches. These bands are firm and compact and are easily lifted from the underlying mesentoderm of the arches as finger-like processes. One or two of these bands of mesectoderm were transplanted at one time and only that part of the band which had reached the mesentoderm of the visceral arches was grafted. Care was always taken not to remove with it the cells of the mesentoderm which are quite white in contrast to the grayish-brown mesectoderm cells. A wound previously prepared in the side of the body of another embryo at about the same stage of development received the transplant. In most cases this

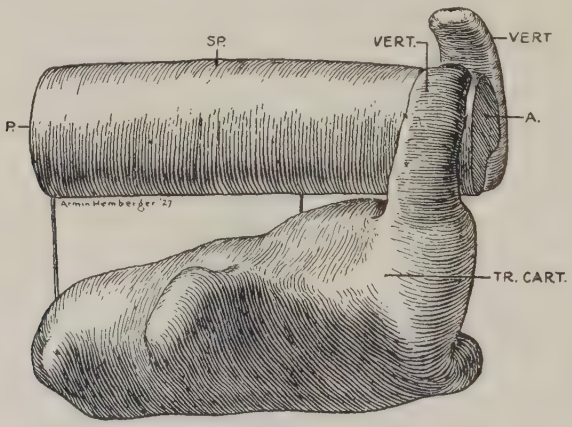

FIG. 2.

Showing from right side a wax reconstruction of a large cartilage (TR. CART.) developed from transplanted mandibular mesectoderm. It is flattended against side of notochord. The right vertebra (VERT.) is fused with its anterodorsal border. SP., spinal cord; A., anterior end; P., posterior end. X 100.

wound was prepared by excising most of the lateral and ventral portions of two or three somites and the overlying ectoderm. In a few cases a site much more ventral to the somites was selected. Into the wound was laid one or two bands of mesectoderm, oriented so that the long axes of the bands were parallel with the long axis of the body of the recipient of the graft. The wound was covered by a piece of ectoderm taken from the belly region of the donor of the transplant. This was allowed to heal in place in the usual manner.[1]

Most of the specimens were killed about 18 days after operation when cartilaginous tissue was well differentiated. Many specimens when sectioned revealed from 1 to 4 cartilages in the area of the transplant. These cartilages vary in size from pieces consisting of only a few cells to bars of cartilage over 500 microns in length (Fig. 2). They are in most cases elongated bars and usually their orientation corresponds to that of the bands of mesectoderm at the time of operation. The position of some of the cartilages and their occasional small size indicates that frequently the bands of mesectoderm cells were disoriented and sometimes broken up at the time of the operation when the glass weights were laid upon the transplant to hold it in position while healing took place.

Some of the cartilages of the transplant lie in loose connective tissue between the ventral portion of the somites and skin (Fig. 3). Others are partially imbedded in portions of the injured somites in the area of operation while many lie near or against the lateral surface of the notochord. The hyoid band of mesectoderm in its normal environment forms a large cartilaginous bar. In the cases

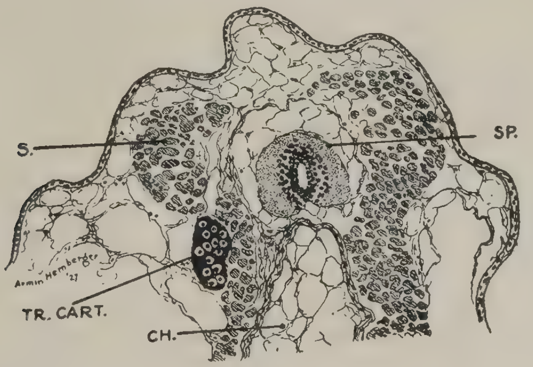

FIG. 3.

Showing a portion of a transverse section passing through one of four cartilages (TR. CART.) developed from grafted hyoid and branchial mesectoderm. It lies lateral to muscle of the injured somite (S.) deep under the skin. SP., spinal cord; CH., notochord. X 66.

where both hyoid and branchial bands of mesectoderm were transplanted it seems most probable that the largest in the group of transplanted cartilages is developed from the hyoid group of mesectoderm cells. In one case (Fig. 2) where only mandibular mesectoderm was transplanted a very large cartilage was developed which lay against the lateral surface of the notochord. Its orientation corresponds to that of the band of mesectoderm at the time of operation. Its size is commensurate with the amount of cartilaginous tissue which the mandibular mesectoderm forms in its normal environment.

In the few cases where the mesectoderm was transplanted to a position in the body wall ventral to the somites no trace of the mesectoderm was found in the form of cartilage. The body wall in this region becomes very thin. It is possible that the transplanted mesectoderm was resorbed at an early stage when it became pushed into the body cavity.

In a few cases the mesectoderm was removed from the branchial and hyoid region and the mesentoderm of these visceral arches plus some of the underlying endoderm was transplanted to somite region. In the region of such transplants loose connective tissue, a few small blood vessels, scattered muscle fibers and some endodermal structures were developed but never any cartilage. This is similar to the conditions found in *Amblystoma* and proves that mesectoderm is the essential tissue for the formation of cartilage in the visceral skeleton.

It is quite clear then that in *Rana palustris* the mesectoderm, migrating over the mesentoderm of the visceral arches, forms considerable cartilaginous tissue and when transplanted to a new environment on the side of the body near the somites, will not form ganglia but cartilage as it does in *Amblystoma punctatum*.

This is a preliminary report.

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[1] Stone, L. S., *J. Exp. Zool.*, 1922, xxxv, 421-496.

[2] Stone, L. S., *J. Exp. Zool.*, 1926, xliv, 95-131.

[3] Stone, L. S., *Anat. Rec.*, 1922, xxiii, 39-40.

3652

Specific Ion Behavior in Gelatin Systems.

MILLARD SMITH.

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Jacques Loeb[1] maintained consistently that specific ion effects played no demonstrable part in the colloidal behavior of proteins, that only the sign of charge and the valence of the ion affect such properties as swelling, viscosity and osmotic pressure, and that the Hofmeister ion series, which implies specific ion effect, is based upon the failure to control the pH of the systems investigated. On the other hand he[2] did not deny the possibility of specific ion effects on the strictly chemical properties of proteins such as solubility, affinity, etc. It is highly probable that many biological phenomena are chemical rather than colloidal in nature, so that investigation of specific ion effects in protein systems may add to the understanding of certain physiological facts.

In order to test the possibility of chemical affinity of gelatin for inorganic ions, experiments were conducted in which dry pieces of sheet ash free gelatin were placed in solutions of known Ph containing equal molecular amounts of HI and HCl, and KI and KCl so that at equilibrium the pH would fall between 3.8 and 4.5, and the concentration of iodide and chloride ions would be 0.1 molar. It was invariably found by chemical analysis that more iodide penetrated the gelatin block than did chloride. It was determined that this was not dependent upon rate of diffusion of Cl^- and I^- ions. In experiments to detect the relative displacibility of chloride and iodide ions from gelatin blocks it was found that iodide possessed more power to displace chloride than vice versa. In order to ascertain whether or not these phenomena were those of chemical affinity, gelatin strips were immersed in KOH solutions containing equal molecular quantities of KI and KCl in such concentration as to make each 0.05 molar, and a final pH of 8.0. At such pH, Loeb has shown that anions do not combine chemically with protein. It was found that more iodide penetrated the gelatin than chloride, even after many days equilibration and with blocks not over 3.0 mm. in thickness. Further experiments showed that this unequal distribution of iodide and chloride ions was dependent upon the presence of gelatin in a gel phase. Thus when 10 cc. of 10 per cent gelatin solution, free from inorganic ions, were placed in a collodion bag, and the latter immersed in a solution 0.05 molar, with respect to iodide and chloride in the form of their potassium salts, and made acid to a pH of 2.2 with equal amounts of 0.1 normal HI and HCl, equal molecular amounts of iodide and chloride were found in the gelatin solution after equilibration.

A series of 10 per cent gelatin solutions was made to contain

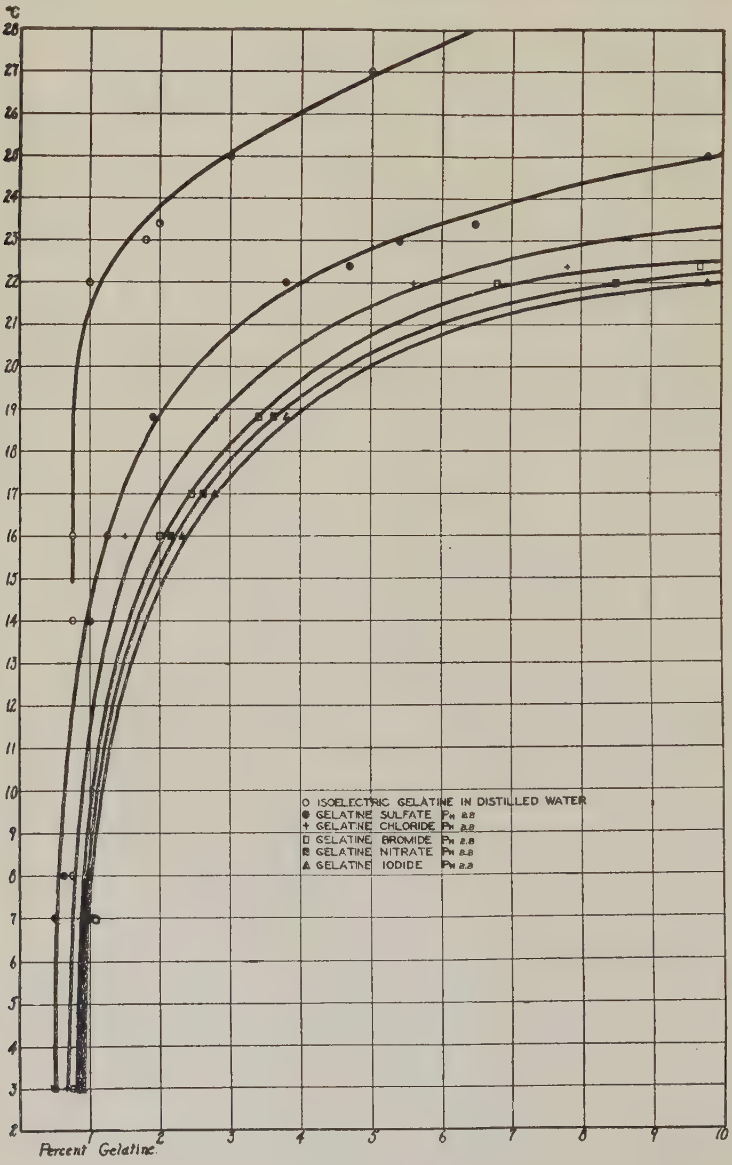

CHART 1.

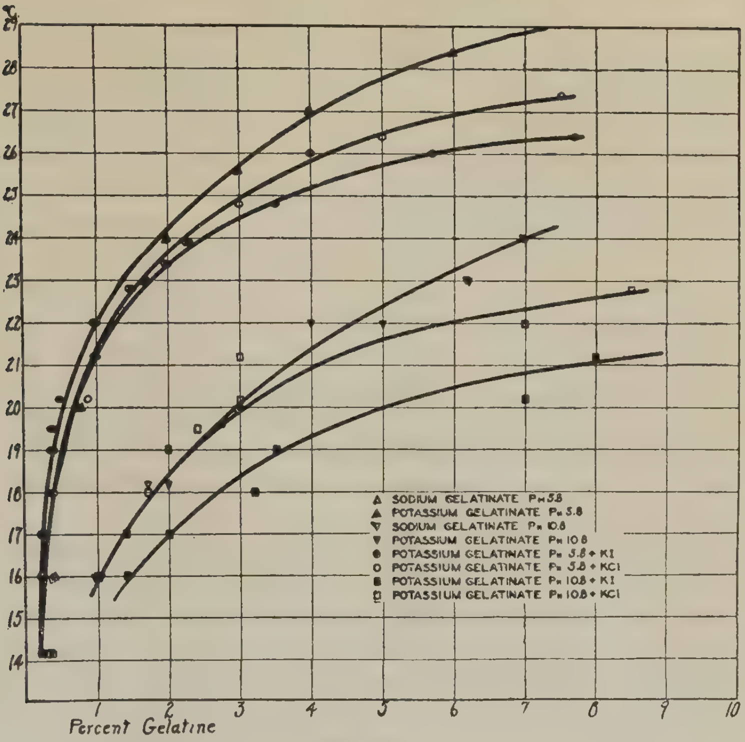

CHART 2.

sufficient HI, HCl, HBr, HNO_3, or H_2SO_4 to make the pH of each solution 2.2. For each of these gelatin solutions a series of 10 test tubes was assembled. Varying amounts of the gelatin solution were placed in the tubes, together with such varying amounts of the corresponding acid solution of pH 2.2 so that the final volume and pH were respectively 5.0 cc. and 2.2 for each tube. Thus five series of different gelatin-acid solutions all of the same pH, but with varying concentrations of gelatin were obtained. These tubes were placed in a thermostat controlled water bath for 24 hours at each temperature, starting at 37° C. In Chart I are shown curves based upon the greatest percentage of gelatin remaining in a fluid state at any given temperature, compared with ash free gelatin in distilled water.

Chart 2 shows similar curves obtained with KOH and NaOH solutions of pH 5.8 and 10.8 and with the additions of KI and KCl in amounts equivalent to the amounts of I^- and Cl^- ions in the series of tubes from which the data for Chart 1 are taken.

These experiments, though preliminary and not at present subject to interpretation, indicate that specific ion effects may occur in the presence of gelatin existing in a phase system.

This is a preliminary report.

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[1] Loeb, Jacques, "Proteins and the Theory of Colloidal Behavior," 2nd edition. 1924. McGraw-Hill Book Company, New York. Pages 107-132.

[2] *Ibid.*, pp. 16-17, 256-257.

3653

Studies on "Salt Ophthalmia" III.

E. V. MC COLLUM, NINA SIMMONDS AND J. ERNESTINE BECKER.

From the Department of Chemical Hygiene, Johns Hopkins University.

Further studies on the production of ophthalmia in rats, induced by feeding a diet containing our salt mixture 20,[1] have established the fact that the ferrous sulphate contained in this salt mixture as the source of iron is responsible for the eye changes observed. We have found that if the diet is made up daily, so that there is but a short time of contact of the ferrous sulphate with the other constituents of the food mixture, animals which have declined and developed ophthalmia under the conditions of our experiments respond with resumption of growth and recovery of the eyes. Other experiments in which facts have been left in intimate contact for a few days with ferrous sulphate have shown that vitamin A is destroyed by this contact.

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[1] McCollum, E. V., Simmonds, N., and Becker, J. Ernestine, *J. Biol. Chem.*, 1922, liii, 313; *ibid.*, 1925, lxiv, 161.

3654

Vitamin Studies XV. Assimilation of Vitamins A and D in Presence of Mineral Oil.*

R. ADAMS DUTCHER, J. O. ELY AND H. E. HONEYWELL.

From the Department of Agricultural and Biological Chemistry, Pennsylvania State College.

In our work on vitamin A it became necessary to dissolve known weights of butter fat and cod liver oil in vitamin-free oils, in order that the feeding of small amounts of vitamin-containing oils might be facilitated. We thought it would be of interest to observe the effect of mineral or paraffin oil on the assimilation of the fat-soluble vitamins, inasmuch as this type of oil should be a solvent for fat-soluble substances. Since the mineral oils are not absorbed in the gastro-intestinal tract, it is to be expected that the mineral oil should remove much of the oil-soluble materials with which it comes in contact.

In the vitamin A studies all rats were placed on the irradiated casein-dextrin ration[1] with daily additions of 0.4 grams of Fleischmann's special dried yeast. When the animals had depleted their storage of vitamin A, which occurred at about the 37th day of the preliminary feeding period, the various oil mixtures were fed separate from the ration in the daily dosages described above. All mineral oil-butter fat mixtures were made in the proportion, by weight, of mineral oil 2.5 to 1.0 of butter fat, 7 drops of the mixture containing .0666 grams of butter fat.

The first group of animals was fed a curative dose carrying .0666 grams of butter fat. None of these animals responded to the mixture, xerophthalmia developed and three of the animals died. At this point the mineral oil was removed and the butter fat feeding was continued. All animals began to grow and the ophthalmic condition disappeared. (See Curve I).

Three animals were given similar treatment except that the mineral oil-butter fat mixture was fed at a 14 drop level, which was equivalent to 4 drops of butter fat weighing .1333 grams. One animal died and the others failed to grow satisfactorily. At the ninth day the mineral oil was removed and the butter fat feeding was continued with an immediate response in growth. (See Curve II).

A third group was fed in the same manner, except that the daily

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* Published by permission of the Director of the Agricultural Experiment Station as Scientific Paper No. 430.

allotment of the oil mixture was increased to 21 drops, which represented 6 drops or 0.2 grams of butter fat. These animals also failed to grow and the mineral oil was again removed and growth was resumed. (See Curve III.)

The fourth group was given the same treatment, using the oil mixture which contained daily additions of .1333 grams of butter fat. These animals were allowed to remain on experiment for 56 days, while the mineral oil was alternately added and removed at stated intervals. The growth curves of the animals rose and fell with the omission and addition of mineral oil. (See Curve IV). To conserve space only representative curves are given.

It is quite evident from the results obtained that mineral oils possess the property of dissolving vitamin A from food materials, thereby depriving the body of this vitamin, even though vitamin A be present in excess of the body needs.

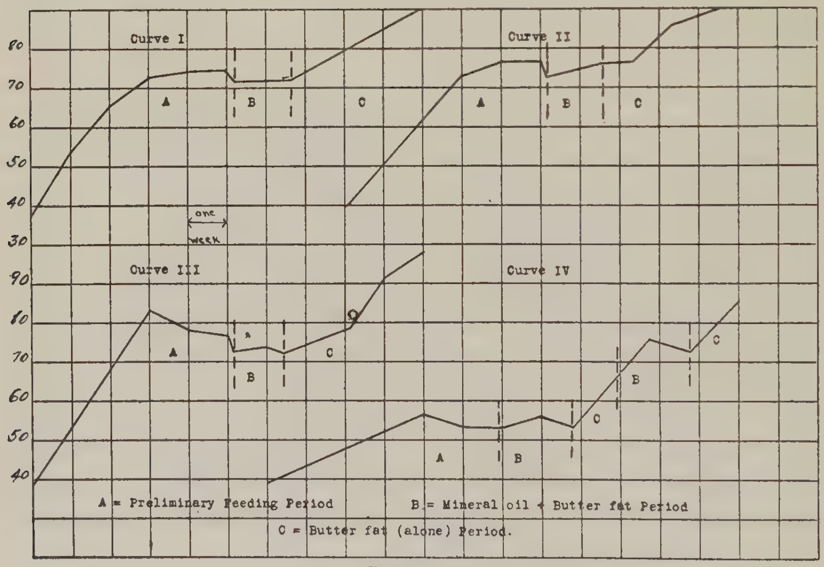

CHART 1.

The fifth group, consisting of 10 rats, was placed on experiment to study the calcifying potency of cod liver oil when fed in the presence of mineral oil. The same technique was followed as described in a previous paper[2] using the Steenbock yellow corn ration.

A mineral oil-cod liver oil mixture was prepared of 3.56 parts (by weight) of mineral oil to 1 part of cod liver oil. Previous experiments with this sample of cod liver oil (4 drops daily) had yielded 41.5 per cent of (femur) bone ash after the animals had subsisted on the Steenbock ration for 21 days. In the study of the effect of mineral oil the animals received daily dosages of 15 drops of the mixture which contained 4 drops of cod liver oil weighing .1180 grams.

When the rats had subsisted for 21 days on the Steenbock ration, and the daily allotment of 15 drops of oil mixture, all animals were chloroformed and the femur bones were dried, extracted with alcohol and ether and ashed. The average percentage of ash obtained was 40.80, which approximates the results we obtain when cod liver oil is fed alone. Line tests were performed on all of the animals and the pictures indicated a degree of calcification identical with that obtained when cod liver oil is fed in the absence of mineral oil, thereby substantiating the results obtained by the ash method.

It is clear that mineral oil may act as a solvent for vitamin A, thereby depleting the ingested foods of their supply of this vitamin, but we have obtained no evidence to show that the calcifying potency of cod liver oil is appreciably affected by the presence of mineral oil. It is entirely possible that increased amounts of mineral oil might show a solvent effect on the antirachitic factor.

Work is now in progress to study the entire matter in greater detail, and with particular reference to the influence of mineral oil upon the digestion of fats and oils. We are also planning to study the effect of diets rich in vitamin deficient fats with the view of determining whether or not undigested glycerides may not have a solvent action on the fat-soluble vitamins in the food and body tissues.

Note: After this paper had been prepared and the work announced at the Rochester meeting of the Federated Societies for Experimental Biology (April 11-16, 1927), the work of Burrows, M. T., and Farr, W. K., appeared in this PROCEEDINGS (xxiv, 719, April, 1927), substantiating the findings described in this paper.

This is a preliminary report.

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[1] Dutcher, R. A., and Kruger, J. H., *J. Biol. Chem.*, 1926, lxix, 277.

[2] Dutcher, R. A., Creighton, M., and Rothrock, H., *J. Biol. Chem.*, 1925, lxvi, 401.

3655

Microbic Dissociation: II. The Tubercle Bacillus.

S. A. PETROFF.

From the Research and Clinical Laboratory, Trudeau Foundation, Trudeau, N. Y.

In a previous communication[1] we called attention to the observation made on microbic dissociation of the tubercle bacillus. Two types of acid-fast organisms, a bovine and an avian type, were described, both of which dissociated into two colonies varying in structural characteristics, *i. e.,* one was rough and irregular, while the other was perfectly smooth and appeared very much like moth balls. The bovine tubercle bacillus (B 1) is a culture of moderate virulence which has been in our possession for many years, and the avian culture was obtained from outside sources.

Further investigations have been made by subculturing the single colonies, with the hope of stabilizing their cultural characteristics. At present we feel that various colonies obtained by subculturing are more stable than those ordinarily obtained with other organisms. In all probability this is due to the fact that the tubercle bacillus develops very slowly, taking weeks for visible colonies to appear. In other words, dissociation may be in proportion to the rate of the multiplication of the organisms.

In this communication we shall describe two distinct acid-fast organisms isolated from the sputum of a patient suffering from pulmonary tuberculosis. These organisms were isolated by the author's method, *i. e.,* equal parts of sputum and 4 per cent sodium hydroxide were mixed, shaken well and incubated for 15 minutes at 37.5° C., then centrifuged and the sediment neutralized with normal hydrochloric acid and inoculated on the surface of Petri plates of gentian-violet-egg medium, which lately has been modified, using the dye in dilution of 1:20,000 instead of 1:10,000. This slight modification of the culture medium seems to be more favorable. Growth appeared after 4 weeks of incubation. Several distinct types of colonies could be fished out and were subcultured.[1]

From this direct seeding 2 colonies were obtained, one perfectly round, smooth and moist which we shall designate as No. 1, and the other, No. 2, dry, rough and very irregular.

Colony No. 1 (Plate No. 1) appeared round, moist, opaque and was easily suspended in salt solution. It grew well in the bottom of glycerol broth and synthetic media, rendering them diffusely turbid in 4 to 5 days. It was impossible to cultivate this organism on the

Plate 1.

Plate 2.

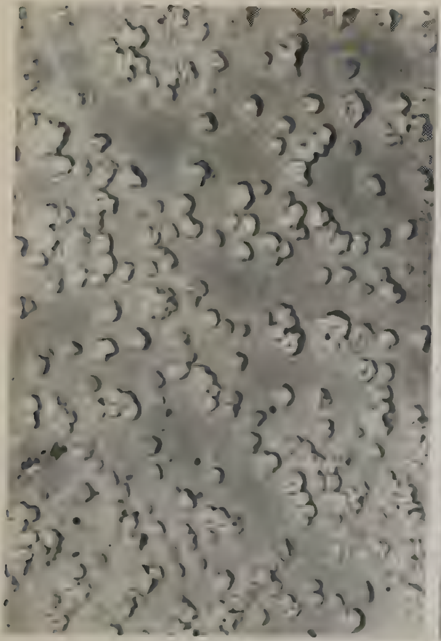

Plate 3.

Plate 4.

All plates actual size.

surface of the fluid medium, as is customary with most acid-fast organisms. When the broth cultures were examined by dark field illumination, only single organisms with an occasional pair could be seen, which stained well by the Ziehl-Neelsen method. There is a probability that this organism can further be dissociated into two distinct types of colonies.

The other colony, No. 2, which was dry, wrinkled, and irregular, was very difficult to suspend in saline. It grew best on the surface of the glycerol broth and had all the cardinal characteristics of other acid-fast organisms. It could further be dissociated into 2 or perhaps 3 types of colonies. One of the colonies (Plate No. 2) was small, flat, with irregular border, very dry and difficult to emulsify. The second colony (Plate No. 3) was also dry but very much more wrinkled, raised and not spreading, while the third colony (Plate No. 4), in all probability, is intermediate.

To our knowledge this is the first time two distinct cultural types of acid-fast organisms have been isolated from a single sputum. One grows homogenously on fluid media, while the growth of the other on the same medium has the ordinary surface growth and appearance considered characteristic of acid-fast organisms.

This is a preliminary report.

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[1] Petroff, S. A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 632.

3656

Correlation of Weight to Counting Method in Determining Number of Tubercle Bacilli.

S. A. PETROFF AND W. STEENKEN, JR.

From the Research and Clinical Laboratory, Trudeau Foundation, Trudeau, N. Y.

In testing the degree of immunity set up by vaccination, either with living or with dead tubercle bacilli, in experimental animals, the lack of uniformity in the selection of the dosage in all probability was responsible to some extent for the discrepancies reported by various investigators.

In calculating such dosage, 3 different methods are in use. Calmette and some of his followers select a dosage based on the calculation that one milligram of moist weight contains 40,000,000 tubercle bacilli. Others in Germany and in this country select their dosage represented in dry weight, using from .001 to .01 milligram, while still others are using the newer direct counting method, which is the most accurate. These various methods have never before been correlated experimentally and no comparison could be made in analyzing the various findings reported in the past.

In this report we shall describe the results obtained in an attempt to determine the number of tubercle bacilli present in one milligram of dry weight. The main object is to bring together all these various methods.

A homogenous type of tubercle bacilli described in the previous paper was used in this experiment. As stated before, this organism grows very readily in the broth and not on the surface like other acid-fast organisms. The suspension was made in physiological salt solution having a pH of 7.8. Phosphate buffer mixtures could not be used for the reason that the salt, being hydroscopic, no complete desiccation could be obtained, and the determinations were not constant. On the other hand, using saline of such a pH, suspensions are easily made and after several filtrations through cotton, approximately 90 per cent represents individual organisms which will not agglutinate spontaneously. One cubic centimeter of the suspension was desiccated to complete dryness. After the weight was determined it was compared with another fraction of the same thick suspension, which was diluted sufficiently so it could be counted by the new counting chamber. The determinations were repeated about six times, from which we calculated that one milligram of dry weight of tubercle bacilli represents approximately 300,000,000 tubercle bacilli. If we take into consideration that moist tubercle bacilli contain 85 per cent of water, then Calmette's figures of 40,000,000 organisms per milligram are about correct. By calculation, and his figures converted from moist to dry weight, the result will be somewhere near 266,000,000 organisms, which closely approaches our actual counting method.

3657

Dissociation of B. Anthracis.

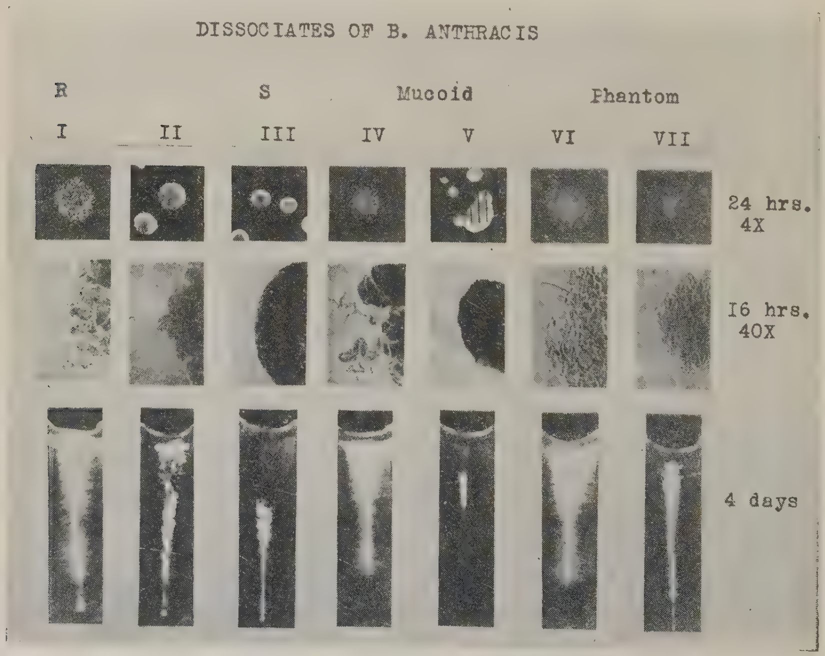
W. J. NUNGESTER. (Introduced by F. G. Novy.)

From the Hygienic Laboratory of the University of Michigan.

Occasional reports of atypical anthrax colonies have been made. These include such observations as those of Preisz,[1, 2] Markoff,[3] Wagner,[4] Gratia,[5] etc. Varying significance has been attached to such atypical forms. Preisz noted these peculiar colonies in vaccine cultures and believed them to be contaminations. After reproducing these colony forms from pure line cultures weakened by growth in broth at 42.5° C., he concluded that they were variants arising from the "weakened" culture. Other workers have generally referred to these modifications merely as variations. Markoff stressed the external environment as influencing variation. Wagner suggested a possible analogy between his "teratologische forms" and the "sogenannten Q-Formen in der Typhus-Coli Gruppe."

In the spring of 1926 we obtained, by plating from broth, a dissociation of an apparently "normal" laboratory stock culture of *B. anthracis* (maintained continuously in the laboratory since 1888) into two colonial types: one was the usual flat, dull anthrax colony, the other a small, round, slightly rough colony. The latter resembled the "B" colony described by Wagner. This dissociation has been repeated on the stock culture purified by over 50 successive single colony isolations.

As a result of these findings we became interested in the behavior of the anthrax bacillus in the light of the modern views on microbic dissociation.[6] Should we consider such atypical colonies as having split from the parent culture and having remained as rather fixed variants? Or was there a closer genetic relationship?


DISSOCIATES OF B. ANTHRACIS

During the last year we have studied further this phenomenon. As a result of aging these first two colony types under various conditions (in plain broth and in broth plus normal rabbit serum, each in 10 cc. and in 300 cc. volumes) we have, in the course of these experiments, noted from time to time the appearance of still other colony forms. At present we are able to report seven distinct colony types, all of which have been reisolated and all of which have been caused to revert to the usual form of anthrax colony and culture. Some of the essential colonial and cultural differences between these forms are shown in the accompanying plate.

Type I gives the growth characteristics of anthrax usually described. A markedly flat, gray, rough colony with the Medusa head appearance. A stab culture in gelatin gives the typical "inverted fir tree" growth.

Type II was our first atypical colony. It forms a smaller, less rough, more raised growth with a less dull surface than that observed in Type I. The colony border is nearly destitute of the typical medusa appearance of Type I. The "inverted fir trees" growth in gelatin is abbreviated.

Type III gives a small, smooth colony with a glistening surface. The border is entirely lacking in Medusa appearance. The gelatin stab gives an abbreviated "inverted fir tree" growth.

Type IV colony is similar to Type I except that it is mucoid. With the aid of a wire it can be made to produce threads 2 to 3 cm. long. This viscosity gives the colony a glistening surface as contrasted to that of Type I.

Type V gives a colony similar to that of Type III with respect to smoothness, but even more markedly mucoid than Type IV colonies. As the mucoid character increases with age the growth becomes transparent. After 16 to 18 hours adjacent colonies tend to coalesce into a slimy mass. In the photograph the markings of a glass scale placed below the plate are visible through the colony.

Type VI colonies are characterized by an extremely faint growth on plain agar. These "phantom colonies" are similar to those described by Soule[7] as occurring in his dissociation of *B. subtilis*. Type VI gives the "inverted fir tree" growth in gelatin stabs.

Type VII reveals colonies similar to those of Type VI but characterized by a diminished diameter of the colony, a more regular border and slightly heavier growth. The growth in a gelatin stab resembles a compact and dense "inverted fir tree".

The direction of the dissociative reactions have not been fully ascertained. However, from the work so far completed there appear to be certain trends of transformation which are more or less constant. Type I may give rise to Type II which then may give rise to Type III. The reverse of this also appears to occur. Starting with Type II we may obtain either Types I or III. Types IV and V, when streaked on agar of a pH of 7.0, tend rapidly to lose their mucoid character and to revert to Type I. The reversion has often been observed as taking place in a single passage on such a medium. Agar having a pH of 7.8 to 8.0 is best suited for maintaining these types.

The relative virulence of the various culture types has also been studied. As a result of over one hundred guinea-pig inoculations, and a similar number of mouse inoculations, it appears that Type I is the most virulent form studied, while Type II stands second. Types III, VI and VII have a lower virulence while Types IV and V have a very low pathogenicity and usually fail to kill guinea-pigs or mice.

Dissociation in the animal body (guinea-pigs and mice) usually occurs when Types IV and V do produce a fatal issue; and under these conditions Types I or II are recovered, along with the type inoculated. A similar dissociation *in vivo* may or may not occur when the inoculum comprises Type VII culture.

Finally, regarding the possible identification of these culture types with the chief culture dissociates now recognized in other bacterial species, we believe that Type I represents the R form of *B. anthracis*. This conclusion is based on general cultural characteristics, and more especially on its stability. Further, all types here represented, tend finally to revert to Type I under conditions of aging. Type III, in view of its cultural characteristics, appears to represent the S form of the anthrax bacillus. Type II, judged by cultural characteristics and virulence, is an intermediate between the R and S. Until further work has been done we prefer not to attempt to correlate further the last four types described to the dissociation scheme.

This is a preliminary report.

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[1] Preisz, H., *Centralbl. f. Bakteriol., I. O.*, 1904, xxxv, 280.

[2] Preisz, H., *ibid.*, 1911, lviii, 510.

[3] Markoff, W. N., *Z. Infekt. d. Haustiere*, 1912, xii, 137.

[4] Wagner, G., *Centralbl. f. Bakteriol., I. O.*, 1920, lxxxiv, 386.

[5] Gratia, A., *Compt. rend. Soc. de biol.*, 1924, xc, 369.

[6] Hadley, P., *J. Infect. Dis.*, 1927, xl, 1.

[7] Soule, M. H., personal communication.

3658

Dependence of Nephropathy Upon Concentration, Kind, and Duration of Feeding of Protein.

L. H. NEWBURGH AND A. C. CURTIS.

From the Department of Internal Medicine, University of Michigan.

In 1919[1] and again in 1923[2] we reported data which led us to conclude that diets high in protein injure the kidneys of the rabbit; and we offered the hypothesis that the injury was caused by the products of the increased amino-acid metabolism. The work was criticized on two scores: (1) The rabbit is a poor test object for experiments of this type; (2) There was no assurance that the diets contained all of the essential accessory factors and the proper distribution of mineral constituents.

Our further work accordingly dealt with the effect of the intravenous injection of various amino-acids on the kidneys of dogs and with the feeding of properly constituted diets, whose protein content varied from 8 per cent to 75 per cent, to rats. The injection experiments[3] showed that the presence in the blood of certain amino-acids in excess of the normal is quickly followed by severe injury of the kidneys.

A preliminary report follows of the data obtained from feeding rats diets containing different amounts of protein.

The diets all contained 3 per cent cod liver oil, 4.5 per cent of the Osborne and Mendel salt mixture, 50 mg. dried yeast extract, lard, corn starch and protein. As the protein increased, the carbohydrate and finally the fat decreased.

Part of the animals represented in the table received casein, the others dried powdered beef as the protein. It is clear that the degree of injury is roughly proportional to the concentration of the protein

TABLE I.

	240 days			480 days		
Protein	Animals	Animals showing casts	Casts in 24 hrs.	Animals	Animals showing casts	Casts in 24 hrs.
Per cent	No.	No.	Av. No.	No.	No.	Av. No.
12	3	0	0	5	1	4
18	11	1	3	10	1	4
25	8	0	0	6	5	83
32	7	5	44	12	12	398
39	9	9	53	10	10	552
75	8	8	2148			

and the duration of the feeding. Histological examination of the kidneys disclosed damage which did not occur before six months, and then only with the higher concentrations of protein.

Furthermore, our data show that the extent of the injury is greatly influenced by the type of protein fed, thus a diet containing 75 per cent of casein fed 8 months caused an average albuminuria of 0.2 per cent and 1100 casts in 24 hours, whereas the same amount of beef proteins for the same length of time, caused 0.6 per cent of albumin and 3200 casts. Finally 11 young rats that ingested the usual amounts of diet containing 75 per cent of lactalbumin, died, without exception, in 7 days or less. All of the urines examined contained large numbers of casts and albumin.

This is a preliminary report.

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[1] Newburgh, L. H., *Arch. Int. Med.*, 1919, xxiv, 359.

[2] Newburgh, L. H., and Clarkson, S., *Arch. Int. Med.*, 1923, xxxii, 850.

[3] Newburgh, L. H., and Marsh, P. L., *Arch. Int. Med.*, 1925, xxxvi, 682.

3659

The Type of Fuel Used in Muscular Exercise.

DAVID RAPPORT AND ELAINE P. RALLI.
From the Department of Physiology, Western Reserve University School of Medicine

In recent papers from the laboratory of A. V. Hill, the moot question of the type of fuel used in muscular exercise has again been raised. The evidence obtained led, in an earlier paper,[1] to the tentative conclusion that "muscular exercise, of such short duration that it does not seriously affect the general metabolism of the body, may be paid for primarily by the oxidation of carbohydrates." In a later communication, Furusawa[2] states that whether the subject be on a carbohydrate or on a fat rich diet, short lived muscular exercise is performed only at the expense of carbohydrate. In more severe exercise, or in exercise of long duration, he postulates a conversion of fat to carbohydrate to replenish the depleted carbohydrate stores. The low respiratory quotients obtained during exercise on a high fat diet by Krogh and Lindhard[3] are, on this conception, due to the latter phenomenon, and would not, presumably, occur in mild exercise. The experiments of Anderson and Lusk,[4] who obtained R. Q.'s approaching those of fat in exercise during starvation, might conceivably, on this point of view, be explained on the ground that carbohydrate was no longer available.

We have recently determined the excess metabolism of exercise in 3 dogs, who were made to run on a horizontal treadmill. The total volume of CO_2 and O_2 exchanged was determined during a preliminary rest period, during the period of exercise, and during the period of recovery until the animal had returned to basal conditions (as shown by the gaseous exchange). The apparatus was accurate to within 2 per cent, as proved by repeated alcohol checks. The maximum deviations in the alcohol check R. Q.'s was from 0.654 to 0.677, the average being 0.664. In other words, the error of the R. Q. determinations in these experiments was hardly more than 0.01.

The dogs were given 3 different diets. The first, which for our purposes, we may consider normal, consisted of about 55 per cent carbohydrate, 30 per cent fat, and 15 per cent protein; the second, 90 per cent fat and 10 per cent protein; and the third, 90 per cent carbohydrate and 10 per cent protein. The latter two diets were not continued for more than 2 days before the experiments were performed. The exercise was carried out in the post-absorptive state, usually about 18 to 20 hours after food.

In some experiments, the animals walked on the treadmill for 45 minutes at from 1.2 to 1.6 miles per hour. Lest this exercise be considered too severe, it was reduced in most of the experiments, and consisted, in the latter, of walking for 15 minutes at the rate of 1.2 miles per hour, obviously a very mild exercise. The animals walked easily, once they had become accustomed to the apparatus. We had, in fact, to increase the speed of the treadmill beyond that of our original intention to permit the animals to walk efficiently.

The results have been summarized in Table I. It is clear that in a rough way the excess R. Q. of exercise and recovery corresponds

TABLE I.

The R. Q. of Muscular Exercise as Affected by Different Diets.

		Basal R. Q.*	Excess R. Q. of exercise and recovery*
Exercise: walking 45 minutes at 1.2—1.6 miles per hour.	‘‘Normal’’ diet	0.82—0.85	0.86—0.95
	High carbohydrate diet	0.91	0.90
	High fat diet	0.74—0.76	0.72—0.74
Exercise: walking 15 minutes at 1.2 miles per hour.	‘‘Normal’’ diet	0.85—0.91	0.79—0.91
	High carbohydrate diet	0.98—1.03	0.96—1.04†
	High fat diet	0.76	0.70—0.78

* Maximum deviations.

† Actual R. Q. of the exercise period in this experiment was 1.008.

to the basal R. Q. On the high carbohydrate diet, the excess R. Q. tends to approach unity, but is not necessarily unity even on this diet. On the "normal" diet, the excess quotient varies usually between 0.80 and 0.90. On the high fat diet, the basal R. Q. is in the neighborhood of 0.76 fairly consistently. The excess R. Q. goes as high as 0.78; in one experiment it was 0.72, in another 0.702, or practically the theoretical quotients for fat. It is difficult to regard this as coincidence, as a fortunate balance between the conversion of fat to carbohydrate with the subsequent oxidation of the latter, plus the burning of extra carbohydrate. Failing proof to the contrary, we are driven to the conclusion that fat is being burned directly. The fact that the R. Q. of a normal animal, exercising on any diet, never falls below approximately the theoretical value for fat, lends added support to this contention.

It appears that in the normal animal the proportion of fat and carbohydrate burned to supply the energy for muscular exercise is a function of the type of diet the animal has been ingesting, and that this holds true not only in severe exercise of long duration, but in a very mild exercise of very short duration as well.

This is a preliminary report.

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[1] Furusawa, K., Hill, A. V., Long, C. N. H., and Lupton, H., *Proc. Roy. Soc.*, 1925, xcvii, 167.

[2] Furusawa, K., *Proc. Roy. Soc.*, 1925, xcviii, 65.

[3] Krogh, A., and Lindhard, J., *Biochem. J.*, 1920, xiv, 290.

[4] Anderson, R. J., and Lusk, G., *J. Biol. Chem.*, 1917, xxxii, 421.

3660

The Influence of Ultra Violet Irradiation of Menotoxin and Pernicious Anemia Toxin.

DAVID I. MACHT.

From the Pharmacological Research Laboratory of Hynson, Westcott & Dunning, Baltimore.

The phytopharmacological studies by the author of menstrual blood and of blood from pernicious anemia patients have revealed that both menotoxin[1] and the toxin present in the blood of pernicious anemia cases[2] are very poisonous for plant protoplasm. A consideration of various physical and chemical properties of these toxins indicates that they are not of the same nature. One of the most striking differences between menotoxin and pernicious anemia toxin is shown by their reaction to treatment with ultra violet rays.

Specimens of blood serum from pernicious anemia cases were examined in regard to their toxicity by the author's method, and their phytotoxic index was determined. Other samples of the same sera were then irradiated with quartz lamps for periods varying from 10 to 40 minutes, and the toxicity of the serum was again determined. Exposure to ultra violet rays for even a short period of time markedly decreased the toxicity of these specimens. This detoxifying effect of ultra violet rays was increased to a high degree by the addition of various sensitizers, of which tetrabromfluorescein was one of the most efficient. By adding small quantities of tetrabromfluorescein or eosin to the serum, the photodynamic effect of the ultra violet rays was greatly intensified, and the sera were detoxified to a higher degree than by the rays alone. This suggested the application of ultra violet irradiation together with eosin to the treatment of clinical cases. The author has already reported a number of successful therapeutic results obtained by administering eosin to patients intravenously and giving them repeated general ultra violet irradiations.

The effect of ultra violet irradiations on menotoxin was found to be very different. Specimens of menstrual serum which exhibited marked toxicity for *Lupinus albus* seedlings were irradiated with a Krohmayer Mercury Lamp in exactly the same way as the specimens of pernicious anemia serum. Such menotoxin specimens showed that they were not at all detoxified by the ultra violet rays, and indeed gave some indication of an increased toxicity. Macht and Lubin[1] stated that there was some evidence pointing to the menotoxin being a cholestrin derivative and more probably an oxycholestrin. The evidence then presented agrees with the recent

TABLE I.
Irradiation with quartz lamp.

No.	Serum Examined	Exposure	Distance	Phytotoxic Index		Remarks
				Before	After	
1.	Pernicious anemia Serum	30 min.	35 cm.	48%	56%	
2.	Pernicious anemia Serum	30 min.	35 cm.	50%	62%	
3.	Pernicious anemia Serum	30 min.	35 cm.	44%	55%	
4.	Pernicious anemia Serum	30 min.	35 cm.	44%	64%	Eosin 1:100,000
5.	Pernicious anemia Serum	30 min.	35 cm.	44%	44%	Eosin 1:100,000 (in the dark)
6.	Menotoxin serum	30 min.	35 cm.	59%	58%	
7.	Menotoxin serum	30 min.	35 cm.	62%	58%	
8.	Menotoxin serum	30 min.	35 cm.	62%	60%	

findings of Okey and Boyden[3] concerning the fluctuations in the blood cholesterin in relation to the menstrual cycle, and also agrees completely with the much earlier findings in the extensive study of Goñalons.[4] It is well known now that ultra violet irradiations produce important changes in the physical and chemical properties of cholestrin, and as pointed out by Beumer,[5] one of the products formed as a result of such irradiation appears to be a substance exhibiting properties attributed by Lifschütz to oxycholestrin.[6] All these facts are of great interest when considered in connection with the effect of ultra violet on menstrual serum here described. The present findings corroborate the original views of the author, that menotoxin is chemically related to oxycholestrin. The table gives illustrations of some of the results obtained.

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[1] Macht, D. I., Lubin, *J. of Pharmacol. and Exp. Therap.*, 1924, xxii, 413.

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3661

Neural and Chemical Control of Ciliated Epithelium.

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(Introduced by V. E. Levine.)

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Practically all workers upon ciliated epithelium have denied that it possesses a nervous control. It is generally held that this type of contractile tissue possesses the property of automaticity, like the heart, digestive organs, urinary bladder, etc., but that, unlike these viscera, it is independent of neural domination. Textbooks of physiology usually omit the question of control in discussing this tissue, and sometimes ignore the subject of ciliated epithelium altogether. Parker and others[1, 2] hold that nervous control of ciliated epithelium is extremely improbable. The histological features of this type of epithelium have been intimately worked out by histologists. A beautiful mechanism, apparently for inter-epithelium coordination, has been described by Grave and Schmidt.[3] Agersborg[4] has recently noted nerve fibers extendnig from the pedal ganglion (of Melibe) to the base of the ciliated columnar epithelium of the ectoderm of the foot. Merton,[5] working on fresh water snail, has recently given evidence for nervous control of ciliated epithelium in this lower form. The weight of authority is, however, decidedly against the view that ciliated epithelium is controlled by means of regulatory nerves.

We have performed many experiments which we believe show conclusively that ciliated epithelium in the vertebrate is definitely under the control of the nervous system. We believe our experiments show just as conclusively that ciliary movement is also subject to chemical control.

For most of this work, we studied the action of ciliated epithelium in the mouth and pharynx of the frog. Our method was as follows: The time in seconds in which this epithelium propelled a particle of cellulose across the field of a binocular microscope was established as a speed norm. Then the rate of travel of the same particle across the same field was determined during and following the stimulation by various methods of the sympathetic and parasympathetic nerves supplying the pharynx.

Electrical stimulation was obtained by means of an inductorium attached to a six volt electric cell, the current used being of just such strength as to give a distinct sensation when applied to the tongue of one of us. The chemical stimuli consisted of sympathetic drugs, ephedrine sulphate and adrenalin, and the parasympathetic drug, pilocarpine.

We made many records of the rate at which the ciliated epithelium of the frog's mouth and pharynx propelled a cellulose particle across the field of the microscope, both before and after stimulation of the sympathetic cord in the region of the brachial plexus. In every case, upon stimulation of the sympathetic, the rate of propulsion was markedly accelerated, in comparison with the rate before stimulation. The resultant increase of speed was 100 to 172.7 per cent.

We obtained a similar speeding up of ciliary action after the injection of epinephrine and ephedrine. These compounds also usually caused the secretion of thick mucus in considerable amounts. The mucus, however, did not prevent the acceleration of speed of the cellulose particle across the microscopic field until a considerable amount of it was secreted.

It is clear from these experiments that ciliated epithelium is speeded up in its action by electrical stimulation of the sympathetic nerves. It is also accelerated by sympatho-mimetic chemical compounds, as adrenalin and ephedrine. Gray[6] showed that the reaction of ciliated epithelial cells to different ions was similar to that of cardiac muscle tissue.

The question arose: Is ciliated epithelium also under the control of the parasymapthetic nervous system? To this question our experiments also give a positive answer.

The point of stimulation of the parasympathetic was the seventh and the ninth cranial nerves as these issue from the *medulla oblongata.* Before stimulation of these nerves the *medulla oblongata* was completely isolated from the rest of the neuraxis by total transverse sections at the cephalic end of the spinal cord and at the cadual end of the midbrain.

Upon establishing again a speed norm, we found in every case upon stimulating the nerves containing parasympathetic fibers to the mouth and pharynx that the rate of ciliary movement was slowed up. The decrease in movement ranged from 78.9 to 114.8 per cent.

After injection of pilocarpine, the rate of speed of the ciliated epithelium was markedly slowed. The rate of slowing ranged from 81.8 to 117.7 per cent.

Sunlight or heat accelerated ciliary movement markedly, and cold decreased it.

Upon examining the action of ciliated epithelium directly through the microscope, we could observe clearly the markedly accelerating and retarding effects of stimulation of the sympathetic and parasympathetic nerves when stimulated electrically and indirectly by the various drugs mentioned above.

Conclusion. The rate of action of ciliated epithelium of the mouth and pharynx of the vertebrate (frog) is increased as a result of electrical stimulation of its sympathetic nerve supply, and decreased as a result of electrical stimulation of its parasympathetic nerve supply.

The rate of ciliary action is also markedly increased by the injection of sympatho-mimetic chemical substances, like epinephrine and ephedrine, and markedly delayed by the injection of the parasympatho-mimetic drug pilocarpine.

This is a preliminary report.

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3662

Growth-Promoting Value of Cod Liver Oil Irradiated by Sunlight and the Mercury Vapor Lamp.

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In order to obtain further evidence regarding the possibility of increasing the antirachitic potency of cod liver oil, we have fed groups of young rats (six each) a standard rachitic ration[1] to which has been added 1 per cent of Norwegian cod liver oil. With one group the cod liver oil was exposed to the direct solar rays, according to the method outlined by Price.[2] A second group received the same amount of cod liver oil, which had been irradiated with a Mercury vapor lamp, at a distance of two feet, for twenty minutes. Control animals were given the non-irradiated cod liver oil. To make sure that the oil, which was obtained from the same source in each case, would be evenly distributed in the ration, it was mixed with 2 per cent of corn oil, a substance which has been shown to contain no antirachitic properties. Two groups of animals receiving the basal rations have been included for comparison. In one case the ration was irradiated with the Mercury vapor lamp in the same manner as the cod liver oil.

Our criteria of the effect of these rations has been the growth of the animals throughout an eight weeks period. The results are summarized in the following table:

TABLE I.
Growth of rats receiving rickets ration with irradiated cod liver oil additions.

Ration	Average initial wt. gm.		Average wt. 8 weeks gm.		Ave. gain per week gm.		Range of gain gm.		Remarks
	♂	♀	♂	♀	♂	♀			
Rickets Control	46	50	62	65	0.2	0.6	0	46	
Rickets ration, irr.	53		177		15.5		124	128	2 animals
Rickets 1% cod oil solar irr.	63	58	138	116	9.3	7.2	44	82	Cod liver oil mixed with corn oil, 2 per cent
Rickets 1% cod oil lamp irr.	54	42	126	100	9	7.3	44	64	Cod liver oil mixed with corn oil, 2 per cent
Rickets 1% cod oil	64	65	134	127	8.5	7.7	46	88	Cod liver oil mixed with corn oil, 2 per cent

It will be observed that the animals receiving the irradiated rations to which no cod liver oil was added made greater gains than those which were given the cod liver oil additions. It would seem, therefore, that the growth stimulation in the animals receiving the cod liver oil was the result of the antirachitic property of the cod liver oil, and not of the Vitamin A contained in the oil.

The gains per week of all the animals receiving the cod liver oil additions are approximately the same. In the group receiving the cod liver oil irradiated by the solar rays, the males gained an average of 9.3 grams per week, while those on the non-irradiated oil gained only 8.5 grams per week. The females, however, receiving the non-irradiated oil made slightly better growth, 7.7 grams as against 7.2 grams in the sun irradiated group. These variations are within the range of error of the method employed. The minimum and maximum gains in all the cod liver oil groups are comparable, but less than that of animals receiving optimum rations or of those on the irradiated rachitic ration. These results are in accord with those of previous workers who have shown that the irradiation of cod liver oil does not increase its antirachitic potency.

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[1] McCollum, E. V., Simmonds, N., Parsons, H. T., Shipley, P. G., and Park, E. C., Ration No. 2249, *J. Biol. Chem.*, 1920, xlv, 333.

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3663

Influence of Feeding Mixture on the Antirachitic Potency of Cod Liver Oil Concentrate.

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From the Child Welfare Research Station, State University of Iowa.

By suitable methods the radio-active substance of cod liver oil can be separated from the oil, and prepared in concentrated form for therapeutic purposes. Recent work by Hart, Steenbock and Hoppert[1] has suggested that such concentrates under certain conditions may fail to be effective. In a study of calcium and phosphorus balances it was observed that the addition of cod liver oil to the ration of milking goats increased the calcium retention.[2] The cod liver oil concentrate (non-saponified fraction), however, was without effect unless it was previously dissolved in oil. The results with the concentrate administered dissolved in oil were comparable to those with the non-saponified cod liver oil.

Since goats are habitually herbivora, taking little or no fat, as such, in their food, it seemed probable that quite different results might be obtained with the omnivora. Drawing conclusions from the work of Hart and Steenbock with goats, one is led to question the availability to the human organism of a concentrate unless given in oil, or in close proximity to a meal which carries fat.

In the study herein reported we have fed young rats a rachitic[4] ration which supplied fat only as it was present in the various cereals of the mixture. To this was added a cod liver oil concentrate equivalent to 1 per cent of cod liver oil. With one group the concentrate was pulverized and dissolved in corn oil (2 per cent of the ration). A second group received the same amount of the concentrate, which was mixed with water and incorporated in the ration. Control animals were given the ration to which was added 1 per cent cod liver oil mixed with corn oil. The rate of growth of the animals on these various food mixtures is summarized in the following table:

TABLE I.
Growth of rats on rickets ration with cod liver oil concentrate.

Ration	Average initial wt. gm.		Ave. wt. 8 weeks gm.		Ave. gain per week gm.		Range of gain gm.		Remarks
	♂	♀	♂	♀	♂	♀			
Rickets Control	46	50	62	65	0.2	0.6	0	46	
Rickets 1% cod oil	64	65	134	127	8.5	7.7	46	88	Cod liver oil mixed with 2% corn oil
Rickets Cod oil con. Equiv to 1% cod oil	58	63	127	123	8.5	7.5	46	76	
Rickets Cod oil con. Equiv to 1% cod oil	70	70	170	139	12.5	8.5	64	122	Con. mixed with 2% corn oil One ♂, 4 young

The animals receiving the cod liver oil and the cod liver oil concentrate mixed with water have averaged the same gain per week, while those receiving the concentrate dissolved in an innocuous oil have made considerably better gains, the males averaging 12.5 grams per week and the females 8.5 grams. On the concentrate mixed with water the males gained only 8.5 grams per week and the females 7.5 grams. The greater gains in the animals receiving the concentrate dissolved in oil, cannot be due to the corn oil, since those receiving the cod liver oil also were given corn oil.

From these results as well as those reported by Hart and Steenbock, it would seem that the most effective method of administering a cod liver oil concentrate is to dissolve it in oil.

This is a complete report.

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[2] Hart, E. B., Steenbock, H., Kletzien, S. W., and Scott, H., *J. Biol. Chem.*, 1927, lxxi, 271.

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3664

Effect on White Blood Cells in Rabbit by Ligation of Common Bile Duct.*

R. B. HOLT. (Introduced by R. S. Cunningham.)

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Jones and Minot[1] have reported that in infectious jaundice, in human beings, the monocytes of the circulating blood are definitely increased in number. Using the supravital technique of Sabin[2] these findings have been corroborated in several cases in the Vanderbilt University Hospital of infectious jaundice. It has further been observed that while the monocytes found in these cases are qualitatively different from those found in the blood of patients having tuberculosis, they nevertheless show more cytoplasmic activity than is customarily the case with the monocytes of the normal individual. The three etiological explanations of these blood changes which seem most probable are: (1) that the rise in monocytes is a direct effect of the invading organism; (2) that it is a specific effect from the retained bile; and (3) that it is a result of some disturbance which has been produced in the liver. If the etiological factor in these changes is dependent upon injury to the liver, the immediate cause may lie either in the production of some injurious agent as a result of the injury to the liver cells, or to disturbed metabolic activity. The following observations are the result of an effort to determine if a specific injury to the liver, caused by a non-infectious process, will produce an increase in the number of the circulating monocytes.

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* This work was assisted by a grant from the Henry Denison Medical Foundation.

Nine rabbits were carefully standardized by making repeated counts of the peripheral blood, using the supra-vital technique. These animals were then operated upon aseptically, and the common bile duct was ligated above the entrance of the duct from the right posterior lobe, so that only this lobe remained functionally active as far as bile drainage was concerned. The blood was studied at least once a day thereafter until the animals died, or until the blood picture had returned to normal. Table I shows the percentages and total numbers of the different types of the white blood cells before and after operation. It will be seen that there was a very marked rise in the monocytes, a slight fall in lymphocytes, and a moderate increase in polymorphonuclear neutrophilic leucocytes. The increase in the neutrophilic leucocytes occurred immediately after the operation, very quickly returned to the pre-operative level and was not maintained as was the rise in the monocytes. This post-operative leucocytosis is well illustrated in Fig. 1, which is taken from a perfectly typical experiment. That the rise in monocytes is the outstanding feature in these experiments is shown both by the curve in Fig. 1, and by the total numbers of the cells found before and after operation (Table I). Obviously these changes in the monocytes were relatively much greater both in the number of cells and in the duration of the elevation than were the changes which occurred in the neutrophilic group. In some of the animals there was a slight rise in lymphocytes immediately following the operation, but this elevation was never maintained for any great length of time, and must be considered as a part of the acute post-operative change which, very significantly, did not include a greater rise in monocytes than was maintained for the whole period during which these cells were so markedly elevated. In those animals which survived for a considerable period of time the monocytes eventually returned to normal and this return probably represented the period at which no further liver injury was being produced. At autopsy the right pos-

TABLE I.

Type of cell	Per cent before operat'n	Per cent after operation	Total No. before operat'n	Total No. after operation
Neutrophils	64%	69%	5129	7603
Basophils	8.4%	3.6%	684.8	400.3
Eosinophils	3.5%	2.3%	273	248.5
Lymphocytes	19%	12%	1551	1267
Monocytes	4.4%	13.1%	367.4	1421.14

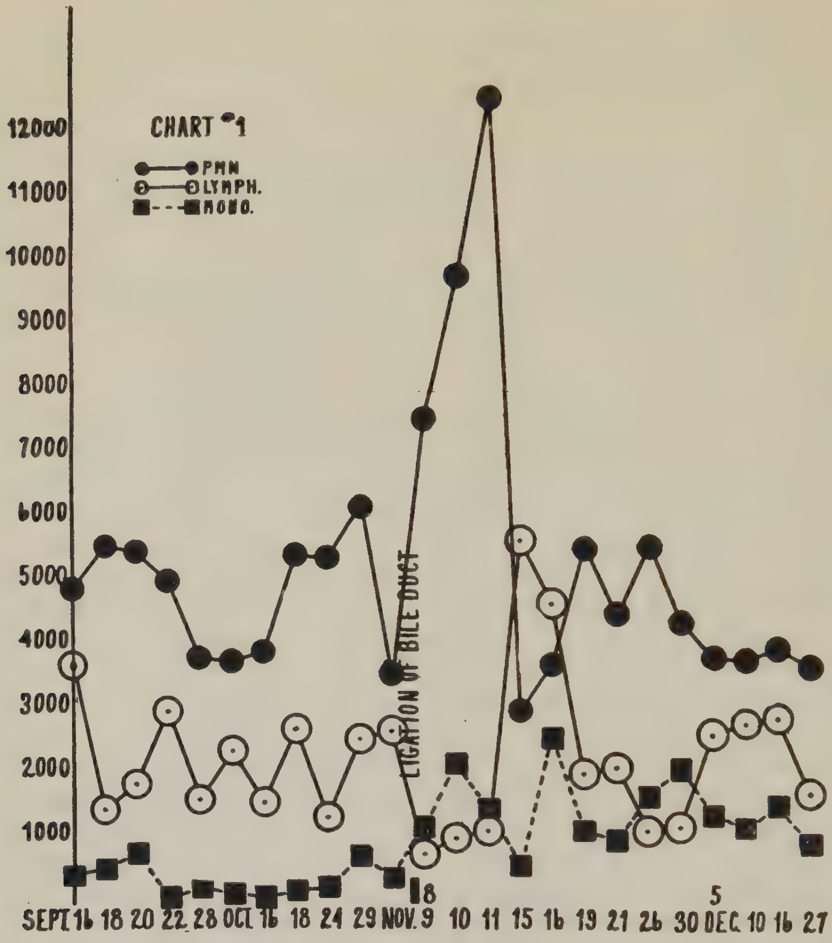

FIG. 1.

terior lobe was hypertrophied but otherwise quite normal, while the remainder of the liver showed the typical degeneration produced by complete biliary obstruction. This type of degeneration has been described so often that it need not be given in detail here. These observations indicate that ligation of the bile ducts is followed by a definite and sustained increase in the number of the circulating monocytes. That an infectious etiology is not operative here is obvious, and the explanation therefore must involve the assumption that the causative factor is either the retained bile or the injury to the liver itself.

In order to determine whether the retained bile was the cause of the blood changes, a few rabbits were given bile (both intravenously and intraperitoneally) and daily counts made of the white blood cells. No changes in the white blood cells were found in these experiments. It must be admitted, however, that the amounts of bile, which we were able to give experimentally, are probably considerably less than would be absorbed as the result of such ligations as were described above. Nevertheless, if the change in monocytes is produced solely as the result of the absorbed bile, we would expect at least a slight increase in such experiments. Therefore we feel justified in concluding that the more probable explanation is that the change in the blood picture is brought about either directly as the result of the liver injury or indirectly by some interference with the metabolic activities of this organ.

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[1] Jones, C. M., and Minot, G. R., *Boston Med. and Surg. J.*, 1923, clxxxix, 531.
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3665

Temperature Effects on Liver as a Result of Lowering Intragastric Temperature With Ice-Water.*

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Considerable work has been done in determining intragastric temperature under various conditions, but comparatively little attention has been paid to the liver. The following experiments were undertaken to determine the immediate temperature effect on the liver following the introduction into the stomach of water at a temperature of 3° C. Dogs were used for the purpose of these experiments and after having been starved for 20 hours were subjected to the following procedure.

Under ether anesthesia the abdomen was opened and thermometers placed as follows: one in the quadrate lobe of the liver, one suspended in the fundus of the stomach cavity, one inserted into a pocket in the stomach wall between the muscularis and the submucosa (except in dog number one this was not done) and of course a thermometer placed in the rectum at the time the anesthetic was started.

In order to introduce the ice-water directly into the stomach cavity a glass tube with the necessary rubber attachments was inserted near the cardiac end. The work was carried out at room temperature, in this case between 30° C. and 31° C., no artificial means being employed to maintain the body heat of the animals.

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* This paper was received too late to be put in regular order.

Temperature readings were taken every 30 seconds for a period of half an hour. The results are readily portrayed in the graphs and may be summarized as follows:

Group No. 1, 500 cc. of water. Dogs Nos. 2, 3, 4, 5, 6.

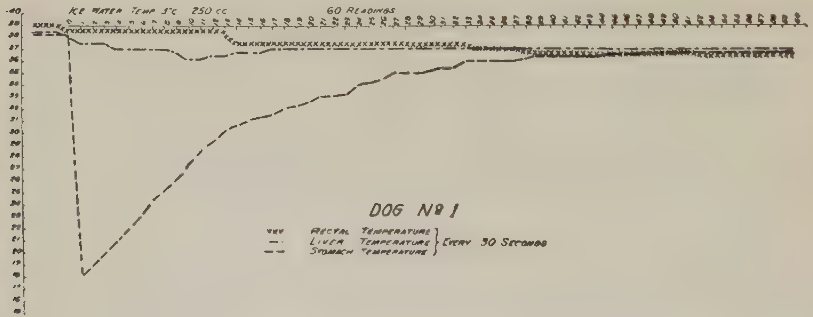
Average drop in rectal temperature	2.45° C.
Average drop in liver temperature	2.25° C.
Average drop in stomach cavity temperature	16.04° C.
Average drop in stomach wall temperature	10.55° C.

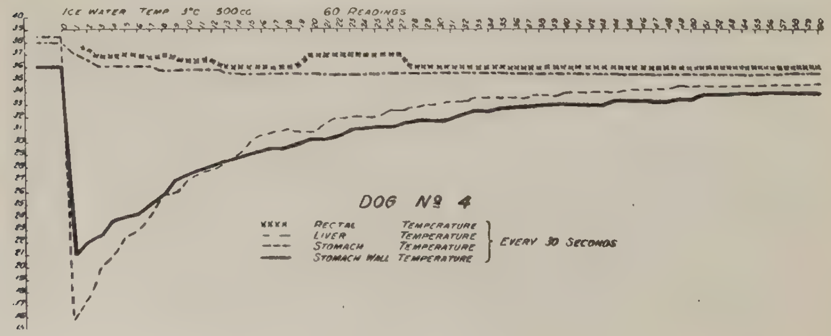
Group No. 2, 250 cc. of water. Dogs Nos. 1, 10, 11, 12.

Average drop in rectal temperature	1.08° C.
Average drop in liver temperature	1.16° C.
Average drop in stomach cavity temperature	15.75° C.
Average drop in stomach wall temperature	6.0° C.

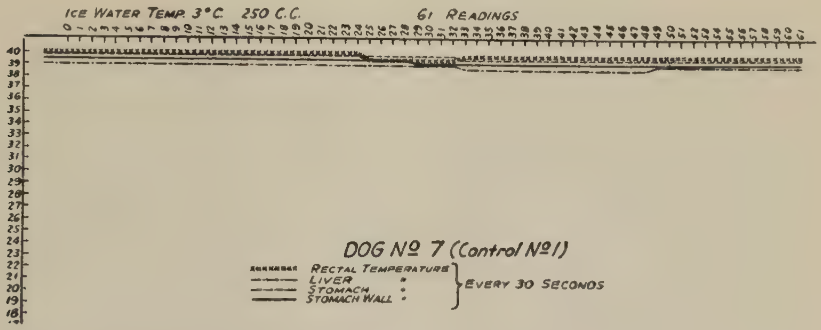
Control Group No. 3, no water. Dogs Nos. 7, 8, 9.

Average drop in rectal temperature	.08° C.
Average drop in liver temperature	.08° C.
Average drop in stomach cavity temperature	.08° C.
Average drop in stomach wall temperature	.08° C.





It will be observed that the operative procedure plus the anesthetic had practically no effect whatever on the body temperatures. Dog No. 7, being control No. 1, shows a very slight drop about half way through the experiment. The other two controls show a slight rise.



From the graphs it will also be seen that in dogs 2, 3, 4, 5, 6 and 11 the temperature of the stomach wall registers lower than the temperature of the stomach cavity. This is probably a local temperature manifestation resulting from an interference of the blood supply incident to making the pocket in the stomach wall for the registration of the stomach wall temperature.

The question arises, what will be the effect on the functional activity of the liver as a result of lowering the liver temperature? To that end it is intended to carry out a series of experiments to measure the bile flow under normal and abnormal conditions.

Conclusion. The liver temperature is affected by lowering the intragastric temperature with ice-water.

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